UNIVERSITY OF SOUTHAMPTON

DIETARY EFFECTS ON PLATELET AGGREGATION

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ABSTRACT

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A major limitation to Single-cell protein (SCP) as a human food is its high nucleic acid content, the purine moiety of which is metabolised to uric acid. Rats given a Fusarium mould (SCP) in diets containing oxonate, a uricase inhibitor, developed elevated plasma and kidney uric acid concentrations after 21 d, which were related to the level of dietary mould.

An association between hyperuricaemia and cardiovascular disease has been suggested, but the results of experimental and epidemiological studies remain equivocal. Platelet aggregation was measured in hyperuricaemic rats and a significant correlation was demonstrated between plasma uric acid concentration and platelet aggregation (maximum rate of aggregation (VAmax) for ADP-induced aggregation $r = 0.88$; thrombin-induced VAmax $r = 0.74$). A linear relationship was shown between the level of dietary mould and platelet aggregation (ADP-induced VAmax $p < 0.01$; thrombin-induced VAmax $p < 0.001$). Furthermore a time lag, exceeding the life-span of rat platelets, was observed between the development of hyperuricaemia and the increase in aggregation.

The effect of other dietary components on platelet aggregation in rats has been examined. Aggregation was reduced progressively when rats were given low-protein diets (50 g casein/kg), but not when 90 or 130 g casein/kg diet was given.

Neither the degree of saturation of dietary fat, nor the level of dietary fat, nor the nature of the dietary sugar (glucose, fructose or sucrose) altered platelet aggregation significantly, although with a high dietary sugar content aggregation tended to be reduced.

The possible interaction between dietary protein, fat and carbohydrate in their effects on platelet aggregation was examined. Platelet

aggregation was substantially reduced in low-protein groups regardless of the dietary sucrose or fat content. Among low-protein groups, aggregation was increased in high-fat groups, but this effect was not observed when the dietary sucrose was also increased. No other effects were observed.

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Abbreviations

cm	Centimetre
d	Days
g	Grammes
G	Acceleration due to gravity
h	Hour
kg	Kilogramme
kJ	Kilojoules
l	Litre
mg	Milligrammes
min	Minutes
ml	Millilitres
SEM	Standard Error of the Mean
wt	Weight

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CHAPTER 1

REVIEW OF THE LITERATURE

Section A

1) Origin of platelets.

Mammalian platelets are formed by subdivision of the cytoplasm of megakaryocytes, which are large cells with polyploid nuclei found in the bone marrow (Wright 1906). The thrombocytopoietic system consists of precursor or stem cells in addition to recognisable megakaryocytes at several stages of maturation. Recognisable megakaryocytes have been classified according to cytoplasmic morphology by light microscopy into three types. Stage I megakaryocytes have basophilic cytoplasm without granulation and a high nuclear-to-cytoplasm ratio. Stage II megakaryocytes are more mature, have more cytoplasm with less basophilia, and some granules may be present. Stage III cells have many granules, a low nuclear-to-cytoplasm ratio, although the nucleus is multilobed, and are the most mature platelet-producing megakaryocytes (Ebbe 1970). The complete disintegration of a single mature megakaryocyte results in the production of several thousand platelets.

Platelets appear to undergo an aging process after they are released from megakaryocytes and the age appears to determine in part their distribution, and their haemostatic effectiveness. The young platelets are usually larger and more dense than older platlets. Platelet life-span varies between species; man 7-14 days, rabbits 2-5 days, and rats 4-5 days. (Ebbe 1971). The bulk of senescent platelets are destroyed in the reticulo-endothelial system of the spleen, liver and bone marrow (Aster 1969).

After their release from the bone marrow, platelets do not enter a pool in which they are evenly distributed throughout the circulation, and the spleen appears to be the major area in which platelet concentration exceeds that of peripheral blood. The size of the splenic pool has been estimated to be about one-third of the total platelet mass in man (Aster 1966). Platelet production from megakaryocytes appears to be controlled by the number of circulating platelets. This control is thought to be mediated by a humoral thrombopoietic stimulating factor (thrombopoietin). However the site of action, the site of production and the chemical nature of thrombopoietin remain unknown (Shreiner and Levin 1973).

ii) Morphology and functional physiology of platelets

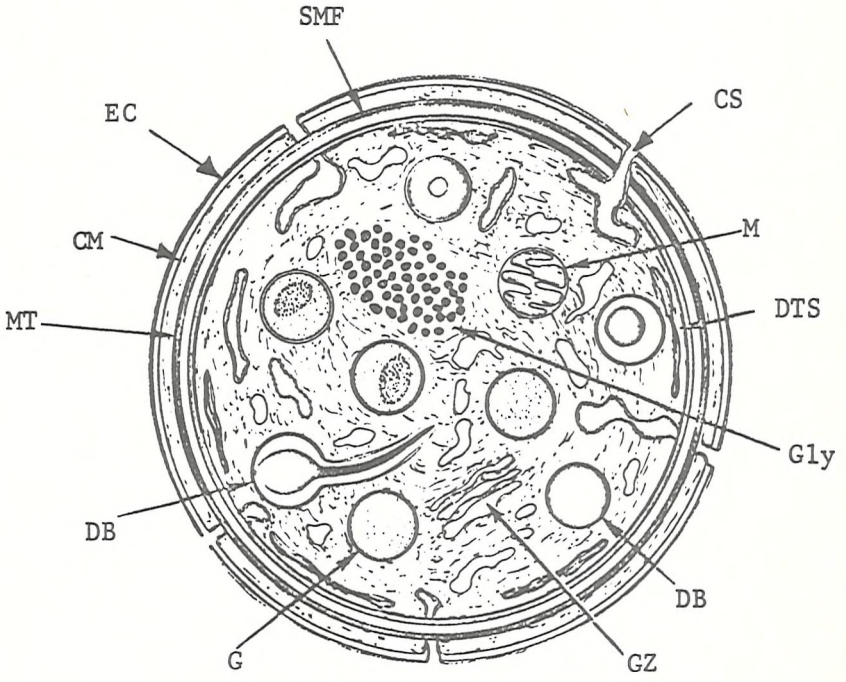
Figure 1 is a diagrammatic representation of a normal human platelet sectioned in an equatorial plane. Platelet anatomy is organized into three functional divisions including the peripheral zone involved in adhesion and aggregation, the sol-gel zone involved in cytoskeletal support and contraction, and the organelle zone involved in storage and secretion (White 1971).

Structural elements of the peripheral zone are the exterior coat, the unit membrane, and a submembrane area containing submembrane filaments. Platelet factor 3, made available during platelet aggregation and involved in the acceleration of blood coagulation, is believed to reside in the lipo-protein rich unit membrane (Marcus, Zucker-Franklin, Safier and Ullman 1966). Submembrane filaments are also included with the circumferential band of microtubules and microfilaments as the three major fibre systems constituting the matrix of the sol-gel zone. Functionally the submembrane filaments may co-operate with circumferential microtubules to maintain the disc shape of normal platelets, play a role in the extrusion and stabilization of pseudopods during shape change, and interact with other elements of the platelet contraction mechanism to enable the platelet to undergo activation (viscous metamorphosis) and clot retraction (White 1971). All three types of fibre contain an actin-like contractile protein, which has ATP-ase activity associated with it, known as thrombosthenin (Luscher and Bettex-Galland 1971).

In the organelle zone numerous granules are found, which are rich in phospholipids and hydrolytic enzymes similar to those in lysosomes (Marcus et al 1966). Other studies have suggested that these granules are storage sites for platelet fibrinogen, thrombosthenin, 5-hydroxytryptamine (5-HT), catecholamines, glycoproteins and a non-metabolic pool of ATP and ADP (Baker, Blaschko and Born 1959; Holmsen, Day and Stormorken 1969; Day, Holmsen and Hovig 1969). Dense bodies are found, but appear to vary in number according to the mammalian species depending on the 5-HT content in the cells. Platelets with abundant 5-HT have many dense granules (Tranzer, Da Prada and Pletscher 1966). It is suggested that dense granules are storage organelles for most of endogenous products secreted by platelets during the release reaction (Holmsen et al 1969). A few platelet mitochondria occur in the organelle zone and are structurally simple, but

Figure 1

Diagrammatic representation of a normal human platelet sectioned in an equatorial plane



CM	unit membrane
CS	open canalicular system
DB	dense bodies
DTS	dense tubular system
EC	exterior coat
G	granules
Gly	glycogen particles
GZ	golgi zone
M	mitochondria
MT	microtubules
SMF	submembrane filaments.

(White 1971)

they contribute significantly to the energy metabolism of the cell (Marcus and Zucker 1965).

Three systems of channels are present in the platelet substance, including the open canalicular system continuous with the cell surface, the dense tubular system associated with the circumferential band of microtubules (Behnke 1967) and a golgi zone found in about 10% of platelets.

Glycogen particles are usually concentrated in masses in the matrix.

iii) Blood coagulation and platelets

Haemostasis is the physiological process, which retains the blood within the vascular system. When there is injury to a blood vessel, the haemostatic process is designed to repair the break and arrest haemorrhage. As a result of injury the blood elements brush against tissue not normally exposed to them, such as connective tissue (basement membrane, elastin and collagen), and materials such as thromboplastin, normally held within the tissue cells. As a result of this contact the haemostatic mechanisms are activated.

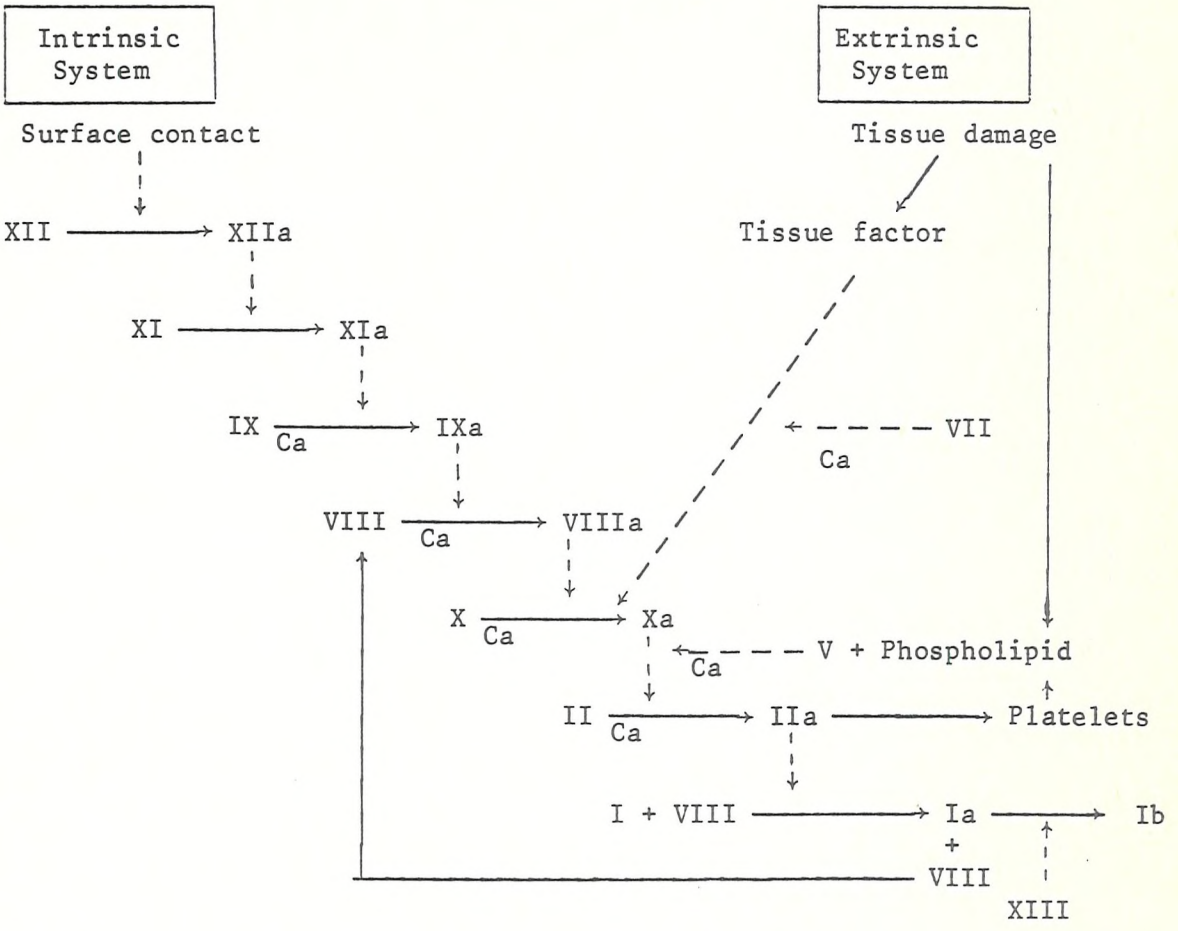
The contribution of platelets towards haemostasis is two-fold. First platelets adhere to the site of damage in a vessel, to exposed extravascular tissues and to each other in aggregates known as haemostatic plugs, which seal any openings. Secondly platelets make available a phospholipid (platelet factor 3), which accelerates plasma coagulation (the conversion of fibrinogen to fibrin). (Born 1972)

The Cascade scheme for blood coagulation was proposed by Macfarlane (1964) and, independently, by Davie and Ratnoff (1964). In these schemes the whole intrinsic pathway from surface contact to fibrin formation is linked by the sequential activation of a train of clotting factors. Each clotting factor (except fibrinogen) exists as an inert proenzyme in the plasma and, after activation, is transformed into a proteolytic enzyme, which activates the next member of the series. This hypothetical system was referred to as an "enzyme cascade" since each molecule of active enzyme releases many molecules of new enzyme from the substrate proenzyme of the next stage.

Figure 2 is a fuller exposition of the cascade scheme (Macfarlane 1965) and also takes into account the Extrinsic system (Biggs 1972).

Figure 2

Cascade scheme of blood coagulation



Factor

XIII Fibrin stabilization factor

XII Hageman's factor

XI Plasma thromboplastin antecedent (PTA)

X Stuart-Power factor

IX Christmas factor

VIII Antihaemophilic factor

VII Proconvertin

V Proaccelerin

IV Calcium

III Thromboplastin; Tissue extract

II Thrombin

I Fibrinogen

Ia Fibrin

Ib Stabilized fibrin

The scheme shows that phospholipid, made available by the platelets (platelet factor 3), is required for the Intrinsic activation of prothrombin. In the Extrinsic system tissue damage activates platelets, causing the platelets to make available this phospholipid for the prothrombin-converting complex.

Later it was shown that there was a requirement for phospholipid, furnished either by the platelets or from the plasma itself, in the reaction of activated factor IX with factor VIII to form factor X activator (factor VIIIa) (Schiffman, Rapaport, Chong 1966).

Platelets have recently been shown to participate in reactions with blood coagulation factors at every stage of intrinsic clotting, from contact activation to fibrin formation. Platelets can trigger intrinsic coagulation by two alternative pathways, the first of which involves factors XII, and XI, and adenosine diphosphate (Walsh 1972a), and the second of which bypasses factor XII providing factor XI and collagen are present (Walsh 1972b) (Figure 3). These appear to occur in unstirred platelet suspensions, where aggregation does not take place, and platelet factor 3 is not made available. Additional evidence (Walsh and Biggs 1972) indicates that subsequent coagulation reactions occur on the platelet surface, where active clotting factors are protected from inactivation by naturally occurring inhibitors. It was these pieces of evidence that prompted Walsh (1974) to postulate a hypothesis in which the events of primary haemostasis (platelet adhesion, aggregation, and release) and blood coagulation are linked. As platelets aggregate to form a haemostatic plug, they provide a protective and catalytic surface for activation of the clotting mechanisms and fibrin formation.

iv) Haemostasis

An outline of the steps involved in haemostasis is shown in Figure 4. Fifteen seconds after bleeding begins, contact between red blood cells and the injured vessel, results in haemolysis of the red blood cells releasing the ADP, which was converted from tri-phosphate, and the partial thromboplastin. The haemo~~philic~~ static factors in plasma interact with the partial thromboplastin to activate plasma prothrombin to thrombin responsible for the conversion of the fibrinogen to fibrin adjacent to red blood cells (Johnson 1971). The concentration of the released ADP and newly formed thrombin both of

Figure 3

Platelet triggering of intrinsic coagulation

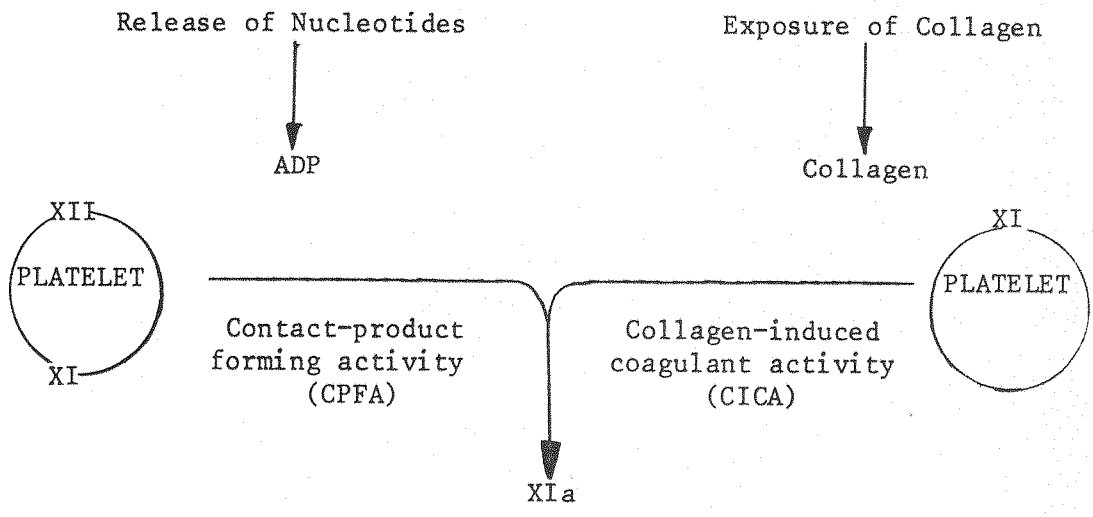
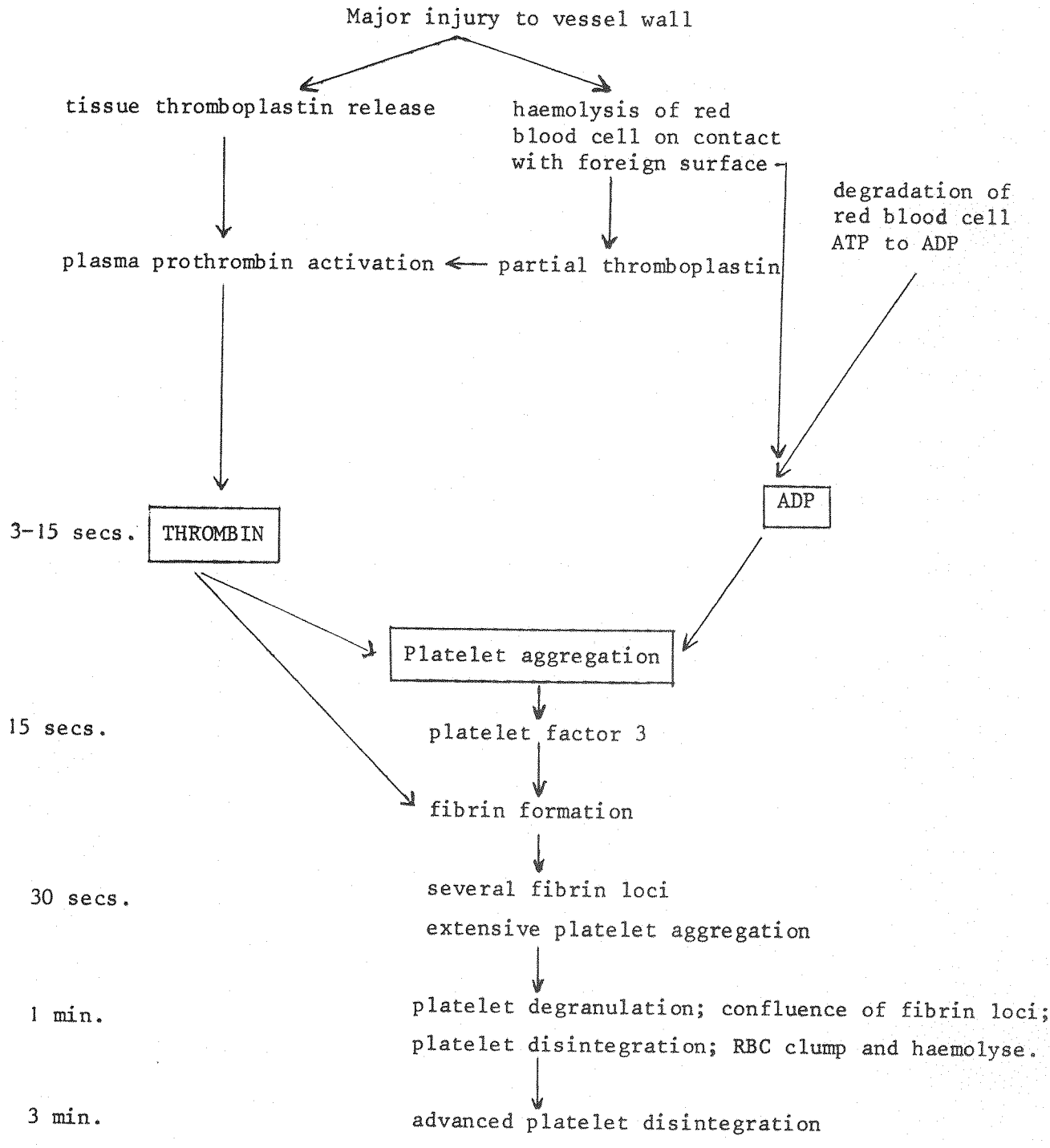


Figure 4

Haemostasis



red blood cells entrapped in fibrin network emanating from platelet aggregates.

which bring about aggregation of platelets is built up steadily. Platelets flow into this area and aggregation begins. The first aggregates to form are small and some attach to the damaged lip of the vessel while some are free in the lumen, as the concentration of these aggregating agents increases in the plasma. These platelets in newly formed aggregates contain many granules.

Thirty seconds after bleeding begins very extensive platelet aggregation has taken place. The haemostatic area has a high concentration of ADP and thrombin and all of the platelets flowing into the turbulent area are drawn into aggregates. Much more fibrin is seen due to release of tissue thromoplastin from exposed smooth muscle. (Kwaan 1969). Interstitial collagen fibres are exposed and are frequently seen adjacent to fibrin fibres. The plasma prothombin is probably activated to thrombin by the changes in plasma factor XII as it comes into contact with collagen. (Niewiarowski 1967).

After one minute many fibrin loci have come together. Opaque areas of fibrin are beginning to form between the aggregated platelets. Platelet aggregates are very large and the platelets are degranulated, having released their α-granule contents including platelet factor 3.

By three minutes when bleeding stops and haemostasis has been achieved, the haemostatic plug, consisting mostly of platelets in capillaries, but of platelets, fibrin, and red blood cells in small arterioles, has completely sealed the gap in the vessel wall. Fibrin fibres have come together in cords made up of several fibres and form a network, which entraps red blood cells. Many platelets have disintegrated in aggregates, but some remain as a nucleus or focal point for the fibrin network. (Johnson 1971)

Clot retraction then occurs in which the coagulum shrinks until it occupies only one-quarter or less of its original volume. Retraction depends on the reaction between ATP in platelets and their contractile protein thrombosthenin, which has ATP-ase activity. (Bettex-Galland and Lüscher 1961) Its function in vivo is uncertain.

v) Thrombosis

Thrombosis is the accidental expression of haemostasis. However, the mechanisms of blood coagulation, formation and release of ADP

from ATP, liberation of material from vessel wall and blood cells into the lumen of the vessel, platelet aggregation and finally arrest of blood flow are evoked in both haemostasis and thrombosis. When these mechanisms are initiated without external bleeding the abnormal situation of thrombosis occurs and the normal flow of blood is obstructed. Both haemostasis and thrombosis are initiated by injury to or disease in the vascular wall. All cellular layers must be severed in haemostasis for external bleeding to occur so that endothelial and smooth muscle cells are ruptured and contribute material to the blood. Connective tissue, including basement membrane, elastin and collagen is also exposed. (Johnson 1971)

In simple thrombosis only the endothelial cells are altered to release enzymes and other cell constituents into the plasma to bring about changes in the red and white blood cells and platelets. Atherosclerosis, which leads to much clinical thrombosis, damages layers deep in the vessel wall, so thromboplastin may be released and connective tissue exposed, thus more closely mimicking the conditions leading to haemostasis. (Duff and McMillan 1951).

The haemostatic blood clot is a structure formed in static blood consisting of red and white blood cells and platelets randomly dispersed in a fibrin network. A thrombus is formed in moving blood and is composed of a head and a tail. The head consists of masses of agglutinated platelets with numerous white cells, but very few red cells and only small amounts of fibrin. Beyond the white head of agglutinated platelets, the red tail consists of fibrin with entrapped red cells. (Poole and French 1961) The distinction between the blood clot and a thrombus is not absolute, since the intermediate grades exist. In the arterial thrombus the major component is usually the platelet head, with the fibrin tail making only a small contribution. The venous thrombus on the other hand consists only of a small platelet head attached to the vessel wall, with a much larger red tail streaming away in the direction of the blood flow. (Douglas 1969)

Platelets play an important role in the pathogenesis of both arterial and venous thrombosis, although there appears to be significant differences in the contribution that platelets make to these varieties of thromboses. In arteries the interaction between platelets and collagen, or collagen-like material in a ruptured atherosclerotic plaque is of primary importance. Other factors involved include the

rate of blood flow, the level of circulating catecholamines, level of ADP inhibitors in the blood and the rapidity with which fibrin is formed to stabilize the platelet thrombus. Changes in blood flow from laminar to non-laminar or turbulent flow due to irregularities in the vessel wall or bifurcations are also important determinants. Increasing evidence occurs that platelet thrombosis occurring on a ruptured atherosclerotic plaque is frequently the culminating event in the development of overt coronary disease. The exposure of fatty atheroma gruel and collagen-rich material to flowing blood results in the rapid development of a platelet thrombus, which in a coronary vessel already narrowed by atheroma may be sufficient to severely reduce or stop blood flow. However, it must be said that the formation and dissolution of thrombi is a highly dynamic process and most thrombi do not persist.

Venous thrombi begin at selective foci, especially valve cusps, where flow conditions are slow and there is the production of local eddy currents. Contributing factors in the development of venous thrombi include the effects of stasis, the local generation of thrombin and its precursors, platelet aggregation with release of platelet contents, and finally conversion of fibrinogen to fibrin. The crucial step appears to be the generation of thrombin with its dual ability to aggregate platelets and to convert fibrinogen to fibrin. Thrombin is generated locally through the intrinsic coagulation mechanism by activated clotting factors, which are not diluted because of local stasis and not cleared by the liver. What causes activation of normally inactive blood clotting factors remains uncertain. (Thomas 1972)

vi) Platelet adhesion

a) Theory

As mentioned the primary events of haemostasis are the adherence of platelets to the site of damage in a vessel, and then to each other to form a haemostatic plug. The former process is known as platelet adhesion, and is defined as the sticking of platelets to a non-platelet surface, and is to be distinguished from the latter process, where there is the sticking of platelets to each other, a process known as platelet aggregation.

It is generally agreed that normal platelets do not react with intact endothelial cells. When small vessels are injured mechanically, thermally, or electrically mural thrombi do not form unless the site has been denuded of endothelial cells. The specific substrate exposed by traumatization of the vessel wall is collagen, the adhesion of platelets to collagen first being shown by Bounameaux in 1959 and Hugues in 1960. Platelets also react, but to a lesser extent with amorphous material (basement membrane) (Tranzer and Baumgartner 1967), to a lesser extent still with microfibrils (Stemerman, Baumgartner, and Spaet 1971), and some workers report adherence to elastin (Hugues 1960, French, Macfarlane, and Sanders 1964).

It appears that many physiological surfaces are available for platelet adherence and that their reactions differ in important respects. Variables affecting adherence include the nature of the reacting material itself, associated intercellular materials that may render the surface less available, and the participation of plasma proteins or other substances that may enhance or retard the adhesion properties of the surface (Spaet and Stemerman 1972). Adhesion is also markedly increased (in vitro) by several substances, which are present in normal vessel walls, such as the catecholamines adrenaline and noradrenaline, and particularly adenosine diphosphate (ADP), a constituent of all cells, which tends to be increased by any form of cellular injury. Another potent agent is thrombin, the formation of which is initiated at injury sites by tissue thromboplastin (Biggs 1972). The effects of all these substances on the platelets, at least in vitro, depends on ADP. In vivo this implies that when a circulating platelet is thrown into contact with collagen, it induces the platelet to adhere until the ADP mechanism comes into play. The effectiveness, however, of agents potentiating adhesion is diminished by blood flow, which dilutes the agents and washes them away. What happens in different vessels is determined by their blood flow.

About the mechanism of adhesion very little is known except that it has been reported to be calcium independent (Hellem and Storaker 1969). It has been reported that an enzyme, collagen:glucosyl transferase, is present on the platelet surface and an enzyme-substrate bond was postulated as being responsible for platelet-collagen adhesion (Jamieson 1971).

b) Tests of platelet adhesion

Most methods for estimation of platelet adhesiveness are based on one of two original methods. Wright's method (Wright 1941) measures the fall in platelet count in citrated or heparinised blood rotated in a glass container under standard conditions, whereas Hellem's original method (Hellem 1960) determines the percentage of platelets retained, when citrated blood is passed through a glass-bead column. "Adhesiveness" is used to describe results obtained with Wright's test and "retention" to describe results obtained with glass-bead columns. Haematocrit, flow velocity, anticoagulant, timing and mixing procedures, age of the glass-bead column, and the type of plastic used influence results of both types of test.

Hellem originally described low retention using platelet-rich plasma and suggested that erythrocytes liberated a substance (factor R), essential for platelet adhesiveness (Hellem 1960). Factor R was subsequently shown to be ADP (Gaardmer, Jonson, Laland, Hellem and Owren 1961). Since the low retention found using platelet-rich plasma could be increased by addition of ADP or erythrocytes prior to testing, the defect was attributed to the absence of erythrocyte-derived ADP, which makes the platelets sticky. However retention is also increased in platelet-rich plasma brought to a normal haematocrit with erythrocyte ghosts even though they do not contain significant amounts of ADP (Zucker et al 1972). Erythrocytes probably promote platelet contact with the glass beads by their effect on flow (Goldsmith 1971). McPherson et al demonstrated that ADP and ATP are released from platelets rather than from erythrocytes (McPherson et al 1974). The released ADP is essential for normal retention, as enzyme systems that destroy ADP markedly inhibit retention.

Salzman suggested that two processes are involved in retention: platelet adhesion to glass and the sticking of platelets to each other. (Salzman 1963). The glass bead column therefore acts as a filter of platelet aggregates and measures a degree of aggregation as well as adhesion.

An ideal test for platelet adhesiveness should distinguish adhesion from aggregation, be carried out on native whole blood, involve a minimum of abrasion and manipulation, and avoid errors

inherent in the platelet count by direct counting of platelets adhering to a test surface.

Recently McPherson and Zucker have developed a method for separating platelet adhesion to glass from subsequent platelet-platelet interactions and therefore enabling them to define the requirement for platelet retention. (McPherson and Zucker 1976). Their findings suggested that platelet adhesion to glass requires fibrinogen but not ADP or von Willebrands factor. This adhesion results in ADP release and a period when platelets stick to each other, but still does not require ADP. However during the period following this ADP is required for further platelet-platelet interactions. This requirement for fibrinogen is in agreement with other reports that a fibrinogen layer must first be adsorbed to glass for platelets to adhere. (Packham, Evans, Glynn and Mustard 1969, Zucker and Vroman 1969). However it has been reported in a patient with Congenital Afibrinogenaemia that adhesion to connective tissue particles was normal, but that behaviour of the patient's platelets on glass surfaces was abnormal. This defect was normalised in vitro by adding small amounts of fibrinogen. (Gugler and Luscher 1965). This exemplifies one of the problems in ascribing platelet behaviour in retention tests to in vivo behaviour.

vii) Platelet aggregation

a) Theory

Adhesion of platelets is followed very rapidly by aggregation. The initial adhesion process cannot really be validly imitated outside the vessel wall so that the mechanism is difficult to investigate experimentally. The process of aggregation, on the other hand, can be observed in vitro by various methods in which it appears to operate much as in vivo. Much more is known, therefore, about aggregation than about adhesion.

It is generally conceded that the chemical mediator of the process in mammals is adenosine diphosphate (ADP) (Born 1962, Born and Cross 1963, Haslam 1964) released from the platelets in response to various extrinsic stimuli. An excellent review of the factors affecting platelet function is that of Mustard and Packham (1970). Stimuli causing ADP release and aggregation include ADP itself (Born and Cross 1963), thrombin (Haslam 1964) collagen and other vessel

wall constituents (see adhesion), and numerous other physiological substances and non-physiological substances (Mustard and Packham 1970), although not all agents are active in all species (Mills 1970).

The aggregation-disaggregation system may be a two phase system. ADP in particular will induce a first phase of aggregation or primary aggregation, the degree of which is dependent on the concentration to which the platelets are exposed. This primary aggregation is followed by disaggregation until, at a critical concentration of the extrinsic ADP, the platelets themselves are induced to release their own ADP to produce a self-propagating irreversible aggregation (Born and Cross 1963, Macmillan 1966). This second phase of aggregation is largely dependent on the source of platelets (Macmillan and Sim 1970), and the concentration of stimulus needed varies with individuals within a species (Macmillan 1966). Adrenaline has also been shown to induce a similar biphasic aggregation of human platelets (Mills and Roberts 1967), although Haslam claimed that ADP is involved in both phases (Haslam 1967).

ADP-induced aggregation is preceded by a morphological change in the platelets, where they change from smooth discs to spheres with pseudopodia of varying lengths protruding from the surface (Macmillan and Oliver 1965). This reaction is very rapid (3-4 seconds) and does not require the cofactors calcium and fibrinogen, which are required for subsequent aggregation (Born 1970, Michal and Born 1971). The rigidity of the protrusions may be due to microfibrils, which can be seen in electron micrographs of the protrusion. The microfibrils, which encircle the platelets beneath the outer membrane and are supposed to provide a cytoskeleton, apparently disappear during the change in shape, possibly they provide the elements of the microfibrils in the protrusion (Born 1970, Born 1972). Microfibrils are composed of a contractile protein, thrombosthenin, that has all the characteristics of actomyosin (Luscher and Bettex-Galland 1971). The change in shape is brought about by other agents except collagen and adrenaline, which have a lag phase associated with them (Biggs 1972).

The change in shape is rapidly followed by primary aggregation. This process has an absolute requirement for calcium and fibrinogen (Born and Cross 1964, Bang 1972). Other plasma proteins including

Hageman factor (factor XII), gamma-globulins, and possibly factor V also promote aggregation (Bang, Heidenreich, and Trygstad 1972). This first phase of aggregation is completely reversible and disaggregation ensues if insufficient aggregating agent is added to cause secondary or irreversible aggregation, or if secondary aggregation does not occur in the species being studied. Some conflict exists as to the cause of disaggregation. It was originally thought to be due to the breakdown of ADP in plasma by enzymic reactions producing inhibitory derivatives (Born and Cross 1963, Haslam 1967, Salzman, Chambers and Neri 1966). However more recently platelet disaggregation has been shown not to be correlated with loss of ADP (Packham, Ardlie and Mustard 1969). Disaggregation has also been shown to require the presence of magnesium ions, although excess can inhibit (Born and Cross 1964).

Above critical concentrations of ADP, secondary aggregation occurs and is associated with the release of platelet endogenous ADP. Secondary aggregation is also associated with the release of other substances, including 5-hydroxytryptamine, adenosine triphosphate, fibrinogen, and platelet factor 4 (anti-heparin factor), as well as the making available of platelet factor 3 for blood coagulation. This release reaction can also be induced by most other aggregating agents including thrombin, connective tissue particles, and adrenaline, the latter which potentiates the action of other agents. Thrombin can in addition release amino-acids, lysosomal enzymes and metallic ions, this effect being known as Release II. (Day and Holmsen 1971). Secondary aggregation is associated with a contraction of aggregates already formed rather than with the formation of new aggregates, and it, in vivo, may increase the effectiveness of the platelet plug as a barrier against further blood loss.

Several hypotheses have been proposed to explain the mechanism of ADP-induced platelet aggregation which are well reviewed (Mustard and Packham 1970, Born 1970).

b) Tests of platelet aggregation

The method, which has provided most information about platelet aggregation is a simple adaptation of a spectrophotometric technique (Born 1962). The method depends on the continuous measurement of

changes in optical density of a suspension of platelets either in plasma or in isotonic saline. When platelets aggregate, due to the addition of an aggregating agent, the optical density decreases and when the aggregates disperse the optical density increases. Shape change is recorded as a rapid small increase in optical density, which is quickly followed by an effect in the opposite direction due to small platelet aggregates forming and then the small aggregates come together to form larger ones.

For further study of shape change the addition of ethylene diamine tetraacetic acid (EDTA) is necessary to prevent aggregation by chelation of calcium ions; also necessary is dilution of the plasma and increased sensitivity (Born 1970).

A method has recently been developed for measuring shape change without inhibiting aggregation (Michal and Born 1971). Advantage is taken of the change in light scattering of the platelet suspension. The aggregometer is modified to record changes in light transmitted through the suspension for aggregation and light scattering at right angles to the incident beam for the shape change. By recording on a twin channel chart recorder, both shape change and aggregation can be monitored.

Recently a new in vivo method for measuring platelet aggregation has been developed by Hornstra and Lussenburg (1975) in which ADP-induced aggregation in circulating arterial blood of rats was determined by means of a "filter-loop technique". This technique is based on the continuous measurement of the blood pressure, in front of and behind a microfilter connected via an aorta-loop to the arterial circulation. Aggregation is induced by infusing ADP in front of the filter. The obstructing aggregates cause a change in the pressure ratio across the filter.

Section B.

i) Single Cell Protein, Gout and Cardiovascular Disease

With the recent interest in the development and use of microbial sources of protein (single cell protein) as a source of food for man, concern is being shown for the nutritional risk factors involved in feeding such materials.

A high nucleic acid content is a characteristic of rapidly growing cells. Micro-organisms contain 8 - 25 g nucleic acid per 100 g of protein. Dietary nucleic acids are depolymerized by nucleases in the pancreatic juices and converted to nucleosides by intestinal enzymes prior to absorption. The purine bases guanine and adenine are metabolised to uric acid in the liver. (Figures 5, 6). This results in increased plasma uric acid and excretion of uric acid in the urine. Since uric acid is sparingly soluble, it can be suspected that an increased plasma uric acid may result in the precipitation of urate in tissue and joints analogous to the condition of gout. Also stones may be formed in the kidneys and bladder. Several human studies have been performed feeding single cell protein material and results showed a significant increase in plasma and urinary uric acid concentrations (Kihlberg 1972). Other human studies involving the feeding of nucleic acid material suggested that the daily intake in healthy adults should not exceed 2 g/d. (Waslien, Calloway and Margen 1968; Edozien, Udo, Young, and Scrimshaw 1970).

Gout is a chronic disease due to an inborn error of purine metabolism. It is characterised by raised blood uric acid concentrations caused either by increased production of uric acid or by interference with its disposal. Although foods rich in purines may initiate an attack of gout, it is generally accepted that they do not cause it. An association has been postulated between gout and vascular disease since the nineteenth century. More recently a number of publications have contributed conflicting information as to the association between serum uric acid concentrations and established coronary heart disease, and its precursors. Gertler, Garn and Levine (1951) noted significantly increased plasma uric acid concentrations in a group of young patients with coronary heart disease. Kohn and Prozan (1959) subsequently

Figure 5

Sources of purines

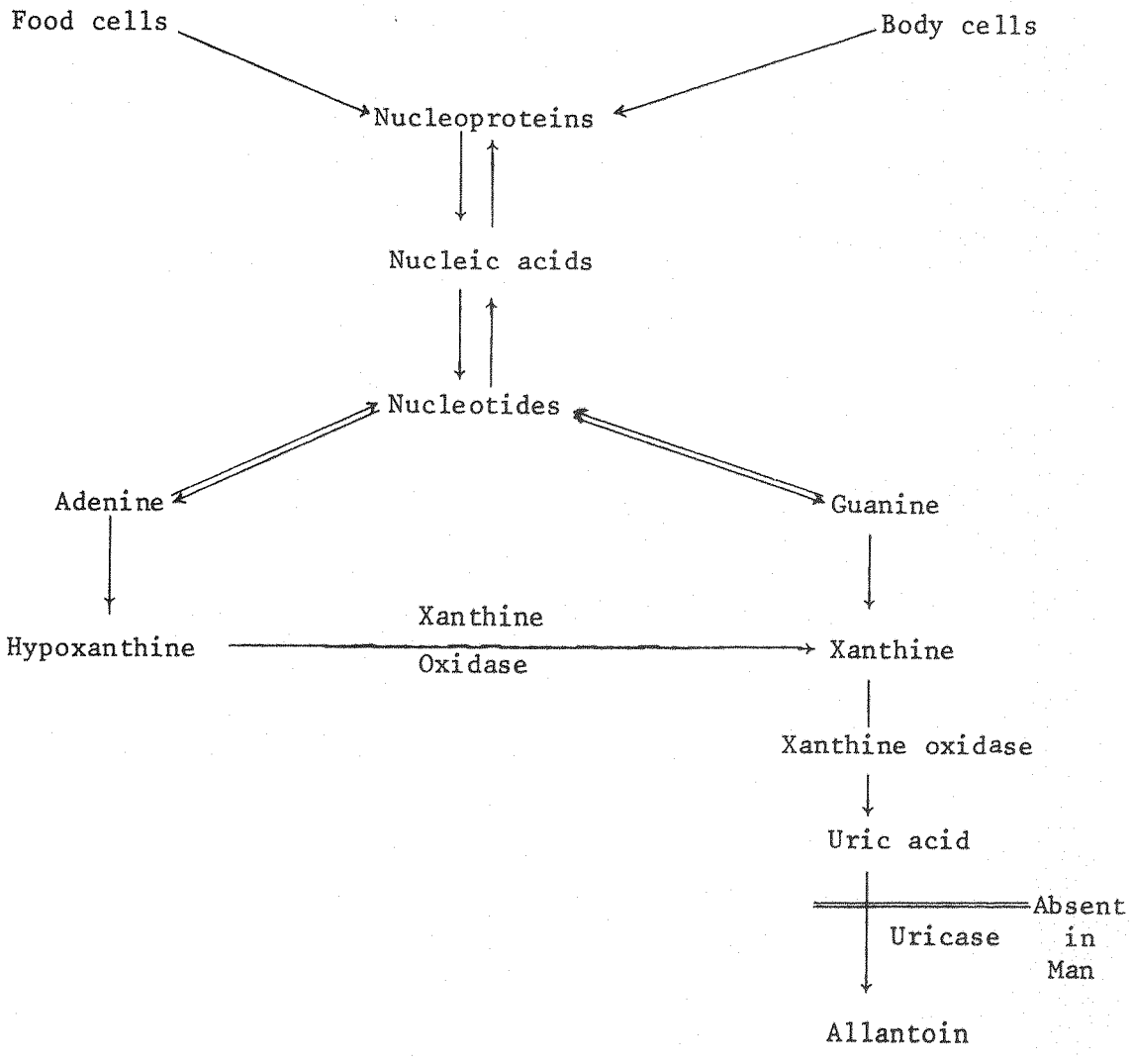
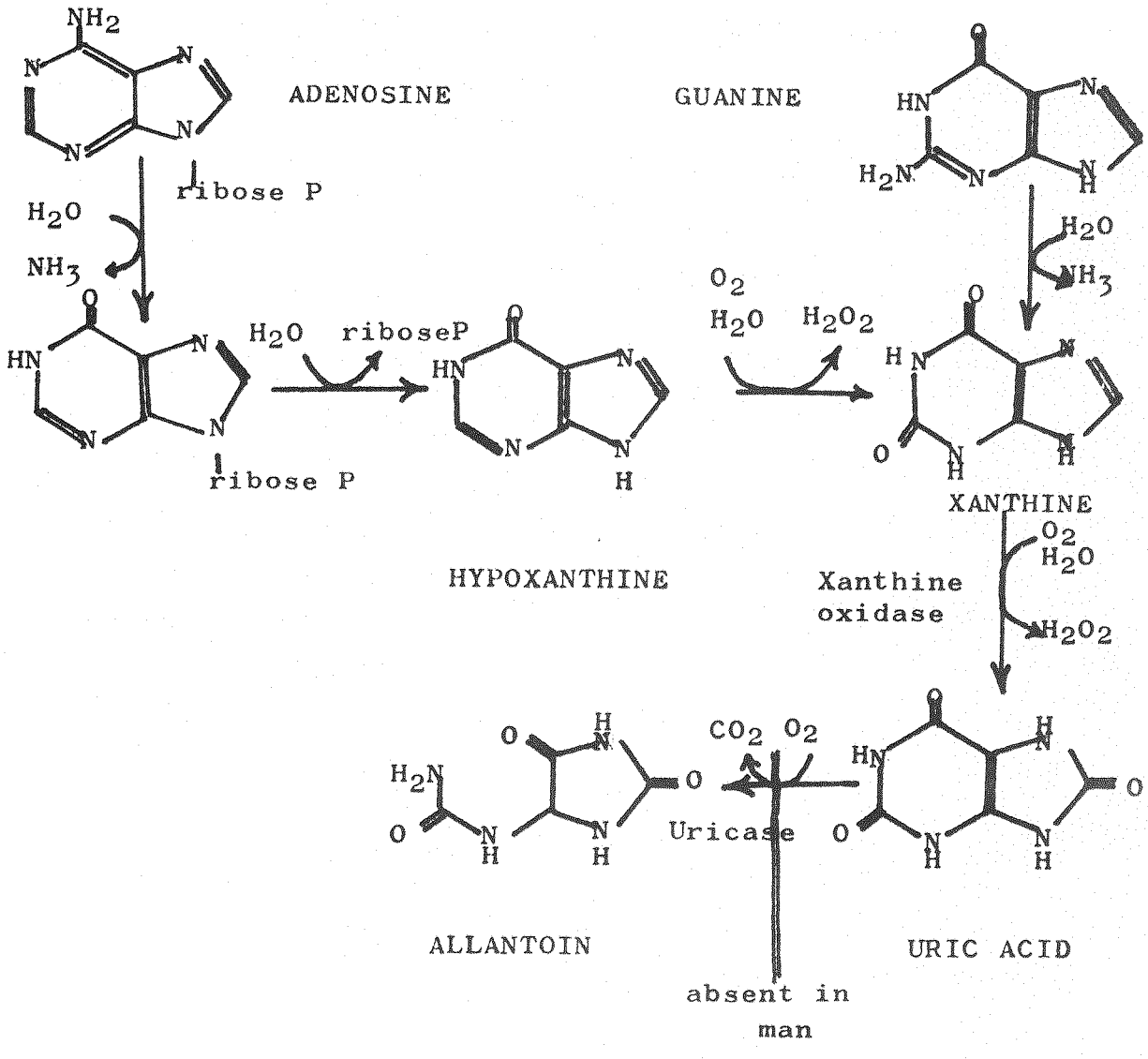


Figure 6

Metabolic route for the degradation of purines.



suggested the use of serum uric acid concentrations in assessing coronary proneness. Several other reports (Ask-Upmark and Adner 1950; Kramer, Perilstein and De Medeiros 1958; Drefus 1960; Spring, Cavusoglu, Chu and Artymowske 1960; Eidlitz 1961; Hansen 1966), have noted the association between hyperuricaemia and the manifestations of atherosclerosis. The original stimulus for some of the studies appeared to be the clinical observation that atherosclerosis and its manifestations, were more common in gouty patients. Yet, at least one authority, based on his longtime study of gout, believes that gouty subjects are no more prone to coronary heart disease than non-gouty persons. (Talbott 1964). A report from a major epidemiological study concluded that there is a higher incidence of coronary heart disease in gouty subjects, but that the association between the risk of coronary heart disease and hyperuricaemia disappears when people with clinically overt gout are removed (Hall 1965). Myers, Epstein, Dodge and Mikkelsen (1968) also found that serum uric acid concentrations of persons with coronary heart disease were not significantly different from the mean of the population studied. Allard and Goulet (1973) studied the relationship between serum uric acid concentration and suspected coronary artery disease as assessed by the severity of lesion shown by performing a coronary arteriogram and found no correlation. The data of Dosman, Crawhall and Klassen (1975) suggested that an abnormality of urate metabolism was present following myocardial infarction This defect was characterised by an increase in the urate pool size and turnover rate regardless of whether the serum urate concentration was increased or not. This might explain the conflict of results in previous studies. However it does not answer the question of whether the abnormalities of uric acid metabolism were related to the acute myocardial infarction, which the patients had sustained, or whether they were part of the metabolic adjustments of the body to the insult of myocardial infarction .

ii) Uric acid effects on platelet function and blood coagulation.

The initial clue to abnormal platelet behaviour in gout came in 1963 when Mustard, Murphy, Ogryzlo and Smythe showed an increased platelet turnover and adhesiveness in gouty patients. When gouty patients were treated with sulfpyrazone (a uricosuric agent), platelet

adhesiveness was decreased. However, in contrast, Darlington, Shaw and Scott (1971) found no significant differences in platelet adhesiveness between gouty and control subjects.

Newland (1968) showed that altered blood concentrations of uric acid influence the incidence of ADP-induced platelet thrombosis in the rat. With hyperuricaemia produced by intravenous uric acid injection, the incidence of thrombosis was significantly increased and with hypouricaemia, produced by intraperitoneal administration of sodium warfarin (a uricosuric agent), the incidence of thrombosis was significantly reduced. It was hypothesized that the blood concentrations of urate influence the stability of ADP-induced platelet thrombi by an effect on the rate of degradation of the ADP in the blood, uric acid itself being a metabolite in the degradation of the adenine nucleotides. In conflict with this hypothesis, Packham, Ardlie and Mustard (1969) showed that platelet disaggregation was not correlated with loss of ADP.

Bluhm and Riddle (1973) studied the in vitro effect of uric acid on platelets at different final uric acid concentrations. They did microscopic determination of the percentage of round and dendritic-type platelets, spread-type platelets, and small platelet aggregates per 100 single consecutive platelets counted. It was found that as the concentration of uric acid was increased, the platelet surface activation was accelerated and the tendency to aggregation increased. In contrast Bologna (1968) showed in vitro that addition of uric acid to citrated human platelet-rich plasma produced a significant fall in ADP-induced platelet aggregation.

Mustard, Glynn, Nishizawa and Packham (1967) reported that uric acid crystals induce release of ADP, AMP and 5-HT from platelets in the presence of gamma-globulins.

In considering the effect of uric acid on blood coagulation factors, Kellermeyer and Breckenbridge (1965) postulated that uric acid may activate Hageman factor (factor XII) and that this could be the mechanism for initiating the inflammatory process in acute gouty arthritis. Glass or similar negatively charged surfaces can activate Hageman factor and these workers showed that urate crystals have a negatively charged surface and can activate Hageman factor in vitro.

In contrast Mustard et al (1963) showed no evidence for abnormal intravascular coagulation in gouty patients. Also Newland's (1968) experiments with warfarin indicated that the uric acid effect on ADP-induced platelet thrombosis was not mediated by alterations in the clotting mechanism.

iii) Fructose-induced hyperuricaemia

Several workers have reported considerably elevated plasma and urinary uric acid concentrations after administration of D-fructose in man either intravenously (Perheentupa and Raivio 1967; Stirpe, Della Corte, Bonnetti, Abbondanza, Abbati and de Stefano 1970; Heuchenkamp and Zollner 1971; Kogut, Roe, Ng and Donnell 1975), or orally (Emmerson 1974). Increased plasma and urinary uric acid and allantoin concentrations have also been reported in rats after intravenous fructose (Maenpaa, Raivio and Kehomaki 1968). These workers suggested the reason being that, as the intracellular level of inorganic phosphate falls due to fructose phosphorylation, the enzyme adenylate deaminase is strongly activated and a transient increase in substrate (AMP) concentration favours the accelerated breakdown of the adenine nucleotides. This results in increased uric acid production.

iv) Use of Uricase inhibitor

It is only in man, higher apes and dalmatian dogs that the enzyme uricase, for the conversion of uric acid to allantoin is absent. Birds also excrete uric acid, but this is produced mainly as a result of surplus amino nitrogen breakdown, whereas in other animals this is excreted as urea.

A method has been devised to simulate hyperuricaemic and hyperuricosuric conditions in more routine laboratory animals involving the feeding of a uricase inhibitor. This is a substance known as oxonic acid, which is an s-triazine and is a competitive inhibitor of uricase (Johnson, Stavric and Chartrand 1969; Kihlberg 1972). This method has also been used in several studies as an experimental model in the study of gouty nephropathy. The feeding to rats of diets supplemented with oxonate and uric acid produced significantly elevated plasma uric acid concentrations, urinary uric acid output, and deposition of uric acid crystals in the kidney. There was also found to be severe nephritis associated with urate deposition. Dietary acetyl

salicylate (aspirin) prevented the inflammatory effects without preventing the deposition of urate (Stavric, Johnson and Grice 1969; Klinenberg, King and Waisman 1971; Waisman, Bluestone and Klinenberg 1973; Bluestone, Waisman and Klinenberg 1973; Klinenberg, Bluestone, Schlosstein, Waisman and Whitehouse 1973; Waisman, Bluestone and Klinenberg 1974).

Section C

Proteins and platelet function

No studies appear to have been reported in the literature concerning the effects of protein feeding on platelet function, although it has been reported that low-protein diets reduced platelet production in rats (Brown 1954). All the blood coagulation factors are proteins. As to whether protein deficiency has any effect on the efficiency of the circulating factors appears not to be known. Fibrinogen, Hageman factor and gamma-globulins are all capable of restoring aggregation capability of washed human platelets. All three of these proteins are cationic in nature and it was postulated that the rate of aggregation is regulated in part by the equilibrium between cationic and anionic proteins in plasma and on the platelet membrane surface (Bang, Heidenreich and Trygstad 1972).

Plasma albumin concentrations are sensitive to levels of protein in the diet and albumin tends to decrease the aggregating effects of the fatty acids (see section on fats and platelet function). This is particularly true when unsaturated fatty acids are studied, since in vitro these acids are bound more rapidly by albumin than are long-chain saturated acids (Hoak, Warner and Connor 1967).

Section D

i) Fats

a) Fats, blood coagulation and platelet function.

During the past 15 years evidence has accumulated, which suggests that eating certain fats in excessive amounts may cause or enhance atherosclerosis and thrombosis. Fatty acids appear to have two potential thrombogenic effects: platelet function and the activation of clotting factors involved in the early stages of blood coagulation.

It is well established that lipids are closely associated with the coagulation process. The activation of Hageman factor and subsequently factor XI can be accelerated by long chain saturated fatty acids (Botti 1963). In this way long chain saturated fatty acids can act as a trigger in the initial stage of intravascular coagulation.

Phospholipids, mainly derived from blood platelets, play an important catalytic influence on at least two reactions in the blood coagulation sequence; namely the interaction between the two antihaemophilic factors VIII and IX, and when activated factor X interacts with factor V.

To what extent excess fat can lead to an enhanced coagulation is still under debate. Kim, Merskey, Deming, Adel, Wolinsky, Clarkson and Lofland (1976) showed that congenitally hyperlipidaemic rats had significantly shorter coagulation and prothrombin times and higher plasma concentrations of coagulation factors II, V, VII, VIII and X than controls. Congenitally hypolipidaemic rats had significantly longer coagulation and prothrombin times and lower plasma concentrations of factors II, V, VII, X and XII and of blood platelets than controls. There was greater thrombosis tendency, as assessed by Hornstra's obliteration time (Hornstra and Vendelmans-Starrenburg 1973) in the hyperlipidaemic group and lesser in the hypolipidaemic group than the controls. These workers also showed that rhesus monkeys with diet-induced hyperlipidaemia had shorter prothrombin times and higher factor X concentrations than controls on normal diet. In agreement with these findings Renaud and Gautheron (1974) found hypercoagulability, atherosclerosis and a high concentration of plasma cholesterol in rabbits fed butter.

With in vitro studies, in general, it appears that studies involving the addition of a variety of lipid materials to clotting systems, show that most of the unsaturated fatty acids have an enhancing effect on blood coagulation. As the saturated fatty acids increase in chain length (from C_8 to C_{22}) they progressively shorten the recalcified clotting time (Verstraete 1972).

Several studies have been reported concerning the effects of lipid feeding on platelet adhesiveness, the most common method being the use of glass bead columns (Hellem 1960). Matteus, Wolf, Cevallos and Holmes (1968) demonstrated increased platelet adhesiveness in rabbits fed a stock diet supplemented with 6% coconut oil and 2% cholesterol for 3-7 weeks, when compared to animals fed on stock diet only. Kloeze (1966a) induced hyperlipidaemia in fasting rats by feeding them daily in a single dose through a stomach tube, a high fat diet containing coconut oil, corn oil, linseed oil, whale oil, soyabean oil, or butterfat for 4 days. Despite large variations in the degree of induced lipidaemia, no significant differences were found in platelet adhesiveness. In 1969 Kloeze, Hautsmuller and Vles fed rabbits three diets; one in which essential fatty acids (linolenic, linoleic and arachidonic acids) were deficient, but containing 40% hydrogenated coconut oil; one in which 10% of the hydrogenated coconut oil was replaced by linolenic acid; and one in which 10% of the hydrogenated coconut oil was replaced by linoleic acid. Results showed no difference in adhesiveness between the diets, although atherosclerosis was more severe and plasma cholesterol concentrations were higher in the hydrogenated coconut oil fed group. Cucuianu, Papilian, Barelüc and Crisnic (1969) reported decreased platelet adhesiveness on infusing sodium palmitate into dogs.

Nordoy (1965) showed an increased platelet adhesiveness in rats given a saturated fat and cholesterol diet. Addition of corn oil (high in oleic, linoleic and palmitic acids) to the diet further increased platelet adhesiveness, whereas addition of linseed oil (high in linolenic acid) normalised the platelet adhesiveness. Owren (1964) also claimed that the addition of linolenic acid to a normal diet reduced platelet adhesiveness. Corn oil and safflower oil, which have a high linoleic acid content, and cod liver oil, which has a high pentaenoic and hexaenoic acid content, had no effect. In 1965a Owren fed rats on a saturated fat diet that was deficient in linolenic acid and reported that they developed increased adhesion-aggregation tendency and an increased incidence of experimental thrombosis in the jugular vein. Supplements of linseed oil reduced adhesiveness and the incidence of thrombosis to the same level as observed on a standard normal diet. However in the same year(1965b) Owren reported that his experiments were not reproducible. Also Berg (1965) found that linolenic acid did not seem to reduce platelet adhesiveness in patients with coronary heart disease. Geill and Dybkaer (1969) in fact found a slight increase in platelet adhesiveness on oral treatment of subjects with linolenic acid.

Composition of Fats

Butterfat	g/100 g total fatty acids
Lauric	2.5
Myristic	11.1
Palmitic	29.0
Stearic	9.2
Oleic	26.7
Linoleic	3.6

Coconut oil	
Lauric	45.4
Myristic	18.0
Palmitic	10.5
Stearic	2.3
Oleic	7.5

Linseed oil	
Palmitic	6.3
Stearic	2.5
Oleic	19.0
Linoleic	24.1
Linolenic	47.4

Soyabean oil	
Lauric	0.2
Myristic	0.1
Palmitic	9.8
Stearic	2.4
Oleic	28.9
Linoleic	50.7
Linolenic	6.5

Whale oil	g/100 g total fatty acids
Lauric	0.2
Myristic	9.3
Palmitic	15.6
Stearic	2.8
Oleic	35.2

Corn oil	
Myristic	1.4
Palmitic	10.3
Stearic	3.0
Oleic	49.6
Linoleic	34.3

Safflower oil	
Myristic	Trace
Palmitic	4.5
Stearic	2.5
Oleic	17.0
Linoleic	76.0

Sunflower-seed oil	
Palmitic	5.6
Stearic	2.2
Oleic	25.1
Linoleic	66.2

(Biology Data Book Vol 1 Federation of American Societies for Experimental Biology. 1972)

Fatty Acids

Saturated

Lauric acid	$CH_3(CH_2)_{10}COOH$
Myristic acid	$CH_3(CH_2)_{12}COOH$
Palmitic acid	$CH_3(CH_2)_{14}COOH$
Stearic acid	$CH_3(CH_2)_{16}COOH$
Behenic acid	$CH_3(CH_2)_{20}COOH$

Unsaturated

Oleic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
Linoleic acid	$CH_3(CH_2)_3(CH_2CH{=}CH)_2(CH_2)_7COOH$
Linolenic acid	$CH_3(CH_2CH{=}CH)_3(CH_2)_7COOH$ - (several isomers)
Arachidonic acid	$CH_3(CH_2)_3(CH_2CH{=}CH)_4(CH_2)_3COOH$

A number of studies have been published concerning the in vitro effects of fatty acids on platelet aggregation. Haslam (1964) reported that the long chain saturated fatty acids caused rapid aggregation of washed human platelets in the presence of calcium ions, when suspended in tris buffer, and suggested that fatty acids mediated these effects by the release of ADP from platelets. Shore and Alpers (1963) found that the addition of stearic acid and other long chain saturated fatty acids to rabbit platelet-rich plasma caused clumping of platelets with the release of 5-HT and histamine. Kerr, Aulay, Pirrie and Bronte-Stewart (1965) found that most sodium salts of free fatty acids dissolved in lecithin, caused aggregation of platelets in platelet-rich plasma. These workers found that linoleic and linolenic acids were ineffective. They also found that linolenic acid inhibited the effects of behenic acid. Ardlie, Kinlough, Glew and Schwartz (1966), using low concentrations of stearic acid, found that it enhanced platelet aggregation in platelet-rich plasma by adrenaline and adenosine triphosphate (ATP), but had no effect by itself. Hoak, Warner and Connor (1967) using microscopic assessment of aggregation found all fatty acids had the ability to produce aggregation when added to washed platelets, but stearic acid was more potent than the unsaturated acids when added to platelet-rich plasma. Calcium was required and aggregation was irreversible. Evidence from a number of in vitro studies including Hoak, indicates that fatty acids bound to albumin have little activity, but it has been postulated that when all the albumin-binding sites become saturated, unbound fatty acids would be present in plasma. Hoak concluded that fatty acids bound to albumin were thrombogenic, but less so than unbound fatty acids.

The variability of results found in the literature concerning the effects of fatty acids on platelet aggregation, probably reflects the different concentrations used and the influence of binding of the fatty acids by plasma albumin. This is particularly true when unsaturated fatty acids are studied, since in vitro these acids are bound more rapidly by albumin than are long chain saturated acids (Hoak, Warner and Connor 1967). More recently Hoak, Spector, Fry and Warner (1970) studied the influence of the free fatty acid/albumin molar ratio on the response of platelets to ADP. Palmitic and stearic acids were most potent, myristic acid produced a moderate enhancement

of the ADP response, and lauric acid had little or no effect. Thus enhancement increased with increasing chain length. The unsaturated free fatty acids, oleic and linoleic acid, caused little or no enhancement even at high molar ratio values.

Positive effects of fat feeding on platelet aggregation have been reported by a number of workers. Renaud, Kinlough and Mustard (1970) observed that platelets from rats fed a diet rich in butter were more sensitive to thrombin-induced platelet aggregation, but not to ADP- or collagen-induced aggregation, than platelets from rats fed a diet rich in corn oil or low in fat. More recently a number of in vivo techniques have been developed to study platelet function, and have been used to study the effects following feeding high fat diets. Hornstra (1971), by measuring the rapidity of thrombosis in aortic loops (obliteration time) in rats, showed that dietary sunflowerseed oil, and the linoleic acid fraction in particular, decreases the thrombogenic properties of rat platelets. It was suggested that this possibly occurs by way of an increased biosynthesis of prostaglandin E_1, which is a highly potent inhibitor of platelet adhesion and aggregation. Hornstra, Chait, Lewis, Turpenien,Karvonen and Vergroesen (1973) using a filtragometer procedure for in vivo measurement of platelet aggregation, found a signifigant reduction in aggregability on feeding male volunteers a high in poly-unsaturated fat (linoleic-acid enriched) and low in saturated fat diet. Although platelet adhesiveness as measured by the rotating bulb method (Wright 1941) showed a slight increase with the diet. These results were confirmed in studies (Fleischman, Justice, Watson and Bierenbaum 1974; Fleischman, Bierenbaum, Justice, Stier, Sullivan and Fleischman 1975) in which the same subjects were placed on both a saturated diet and a linoleic acid-enriched diet so that comparisons were made within an individual rather than between independent groups. In contrast with these findings Nordoy and Rodset (1971) reported insignificant changes in platelet aggregation in men fed soyabean oil (high in linolenic acid) or oleum vegetable oil (trioleate). Also McGregor (1974a)found reduced rate of aggregation and rate of shape change in rats red hydrogenated coconut oil.

b) Prostaglandins and platelet aggregation

The strong inhibitory action of prostaglandin E_1 (PGE_1) on ADP-induced platelet aggregation in rat, pig and human platelet-rich

plasma was first reported by Kloeze (1967), an observation which has been confirmed subsequently. It has been shown also that PGE_1 inhibits the aggregating action of platelets induced by a large number of other compounds including thrombin, collagen, noradrenaline, ATP, 5-HT, antigen-antibody complexes and γ-globulin coated polystyrene particles (Emmons, Hampton, Harrison, Honour and Mitchell 1967; Chandrasekhar 1967; Kinlough-Rathbone, Packham and Mustard 1970). When platelets, which had been incubated with PGE_1, were washed and resuspended in fresh medium, they aggregated normally in response to ADP and, since PGE_1 was not taken up by the platelets, it was suggested that PGE_1 must be in the ambient fluid to exert its effect (Kinlough-Rathbone et al 1970). Similar inhibitory effects of PGE_1 have been demonstrated in vivo. In rats given a prior intravenous injection of PGE_1, thrombocytopenia was inhibited and the LD50 increased, following the intravenous administration of ADP, the PGE_1 having inhibited the platelet thrombi, which are normally formed in animals dying after an injection of ADP. (Kloeze 1970).

There is less agreement about the effects of prostaglandin E_2 (PGE_2) upon platelet aggregation. The discrepancy in results may be explained by some of the preparations of PGE_2 being contaminated with small concentrations of PGE_1 (Kloeze 1969), since it is very difficult to purify the preparations of PGE_2 completely. As increasingly purified preparations of PGE_2 have been produced the weight of evidence now favours PGE_2 having a weak stimulatory effect on platelet aggregation.

The mechanism whereby PGE_1 inhibits ADP-induced aggregation is not yet fully understood, but the most widely held hypothesis is that PGE_1 exerts its influence on platelet aggregation by increasing the intracellular concentration of 3'5' cyclic AMP (c AMP). In this system c AMP is formed from ATP by the action of adenylate cyclase, an enzyme located in the platelet cell membrane, which is sensitive to various platelet aggregating agents. The enzyme phosphodiesterase can destroy c AMP, so that agents, which inhibit or potentiate phosphodiesterase can influence levels of c AMP within the platelets (Salzman 1972). There is evidence to suggest that substances, which inhibit platelet aggregation initiate a rise in c AMP and that those which cause platelet aggregation result in a fall in c AMP (Salzman and Levine 1971).

Recent studies have shown that PGE_2 and $PGF_{2\alpha}$ are formed during blood clotting and platelet aggregation and that these prostaglandins

are released by the platelets. (Silver, Smith, Ingerman and Koscis 1972; Smith, Ingerman, Kocsis and Silver 1973). These observations when considered with the stimulatory effect that PGE_2 is shown to have on platelet aggregation raise the possibility that PGE_2 and $PGF_{2\alpha}$ may play a part in normal platelet aggregation. Alternatively, precursors of these prostaglandins may be important, since arachidonic acid, a precursor of PGE_2 and $PGF_{2\alpha}$ (Fig. 7), is capable of stimulating platelet aggregation. An endoperoxide intermediate (PGR_2), which is formed from arachidonic acid and is readily converted into PGE_2 or $PGF_{2\alpha}$ (Fig. 8), has been shown to be released during platelet aggregation induced by arachidonic acid, collagen and adrenaline and appears outside the platelet before collagen-induced aggregation occurs and may therefore be the trigger to the platelet release reaction. (Smith, Ingerman, Kocsis and Silver 1974). Willis (1974a) proposed that PGR_2 may serve as a trigger for arterial thrombosis, since aspirin, which inhibits the conversion of arachidonic acid to PGR_2 and PGE_2 (Fig. 8) (Willis 1974b), has antithrombotic properties in laboratory animal models of arterial thrombosis. Willis, Vane, Kuhn, Scott and Petrin (1974) suggested that platelet biosynthesis of PGR_2 and PGE_2 mediated, in part or in full, irreversible platelet aggregation and the release reaction induced by collagen, thrombin, arachidonic acid, adrenaline or ADP.

In contrast the corresponding endoperoxide intermediate (PGR_1) formed from dihomo-γ-linolenate during the biosynthesis of PGE_1 and $PGF_{1\alpha}$ (Fig. 8) (Nugteren and Hazelhof 1973) has no effect on platelet aggregation (Willis et al 1974). In addition PGE_1 is a very potent inhibitor of platelet aggregation (Kloeze 1967). However no detectable biosynthesis of PGR_1 or PGE_1 occurs in platelets due to a demonstrable lack of the dihomo-γ-linolenate substrate (Marcus, Ullman and Safier 1969). Oral feeding of dihomo- -linolenate to rats led to its accumulation in plasma and platelet lipids, so that it can compete with arachidonic acid for PG synthetase, which redirects PG biosynthesis thus producing PGR_1 at the expense of PGR_2 (Fig. 8) (Willis, Comai, Kuhn and Paulsrud 1974). Also PGE_1 is produced, which inhibits aggregation at the expense of PGE_2, which potentiates PGR_2-induced aggregation.

A possible explanation to the finding of Hornstra (1973) of the antithrombotic effect attributed to dietary linoleic acid, could be an

Figure 7

Prostaglandin biosynthesis

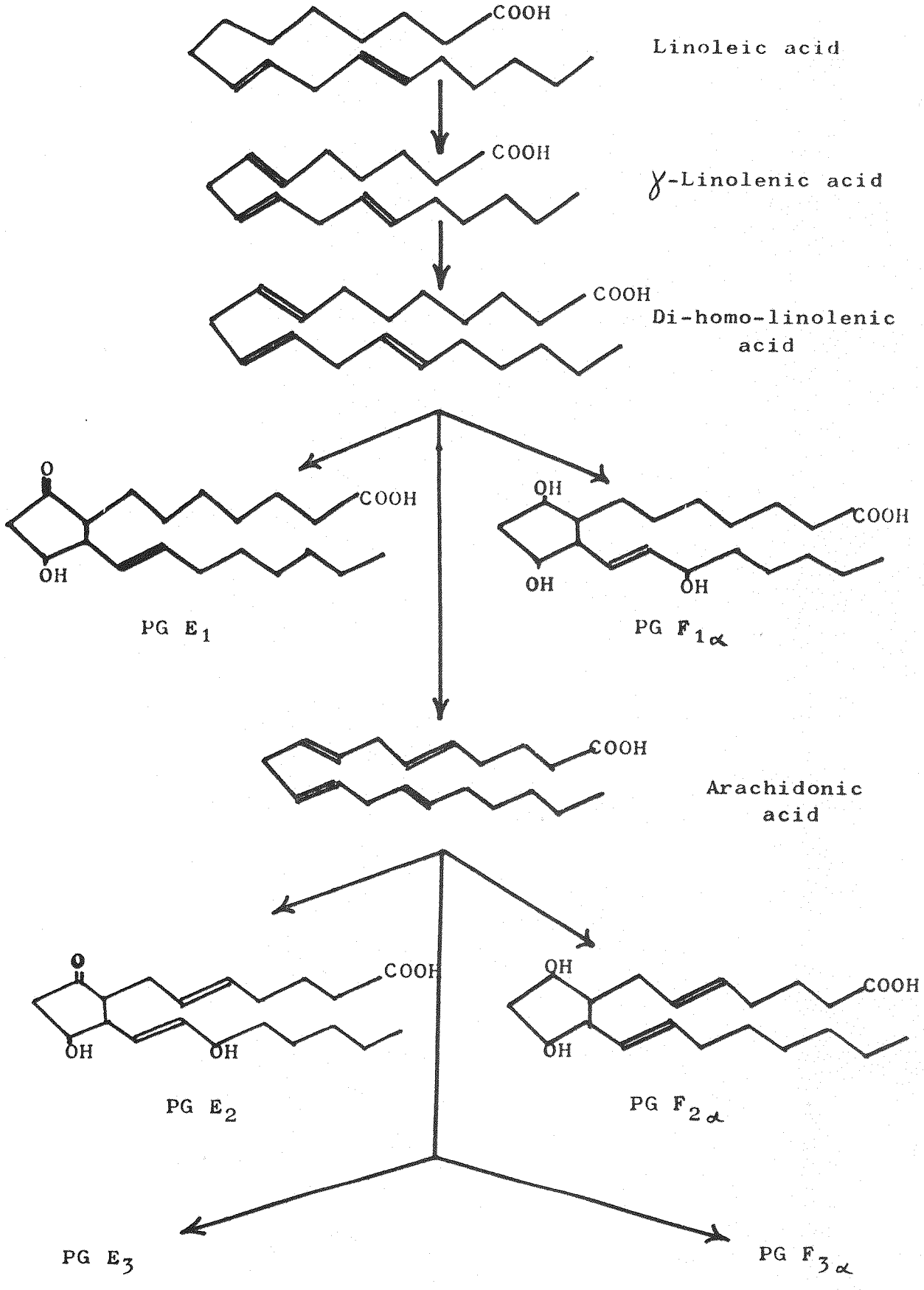
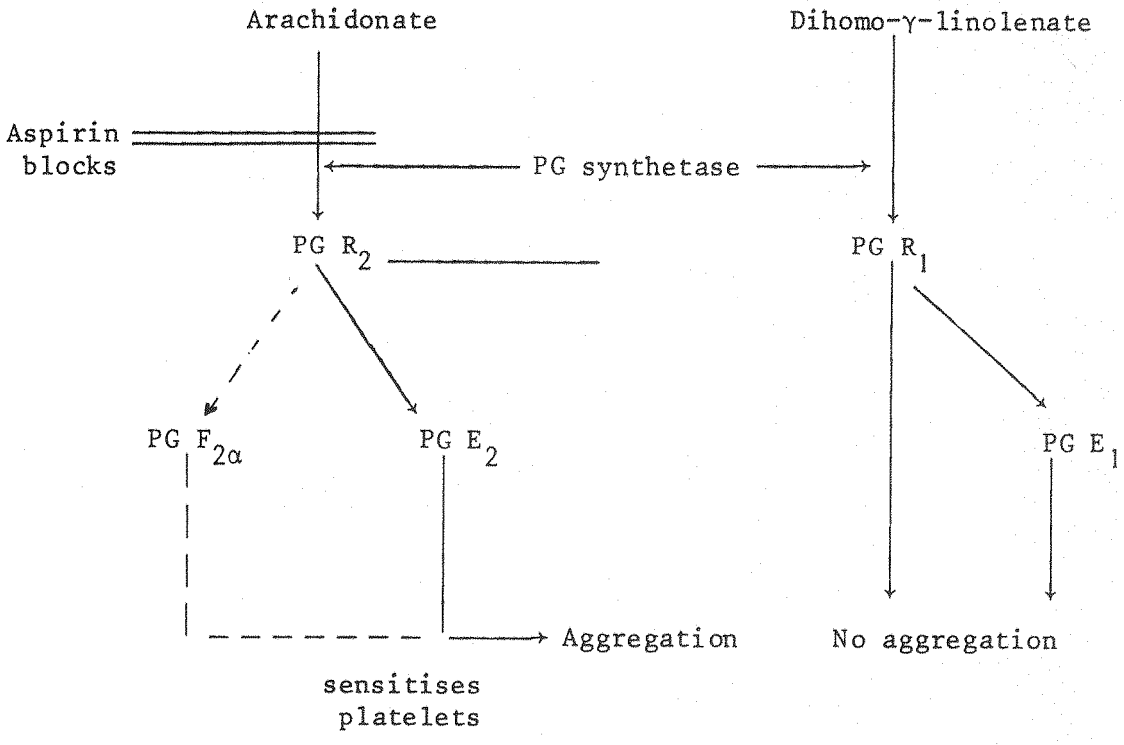


Figure 8

Outline of the mechanism through which orally ingested dihomo-γ-linolenate could inhibit platelet aggregation and hence arterial thrombosis



increased biosynthesis of PGE_1, being a potent inhibitor of platelet aggregation. Since the feeding of increasing amounts of linoleic acid does not result in increasing concentrations of arachidonic acid in platelet cell membranes, it is possible that the observed increased urinary excretion of PG metabolites resulting from diets rich in linoleic acid (Vergroesen and Gottenbos 1975),is indicative of a stimulated PGE_1 biosynthesis from dihomo-γ-linolenic acid.

ii) Carbohydrate and platelet function

It has been suggested that high levels of sucrose in the diet may play an important role in the aetiology of ischaemic heart disease (Yudkin 1957; Yudkin and Roddy 1964; Yudkin 1972). Szanto and Yudkin (1969) found that in human volunteers eating a diet rich in sucrose, the plasma insulin concentration, the body weight and platelet stickiness, as measured by Wright's rotating bulb method (1941) were all increased, in one-third of the volunteers. Later it was found that both serum insulin concentration and platelet adhesiveness correlated significantly with sucrose intake in men with peripheral vascular disease, but not in men without this disease. (Yudkin, Szanto and Kakkar 1969; Yudkin and Szanto 1970). In 1970 Szanto and Yudkin used the measurement of electrophoretic mobility of platelets in the presence of ADP (Hampton and Mitchell 1969) as a test of platelet function. Subjects, who showed "sucrose-induced hyperinsulinism" had, after feeding a sucrose diet for 14 days, a pattern of electrophoretic behaviour of platelets in the presence of ADP; that is characteristic of individuals with atherosclerosis. They postulated that hyperinsulinism is an early manifestation of atherosclerosis, that only a portion of the population show sucrose-induced hyperinsulinism, and that only these will be susceptible to the effect of dietary sucrose in producing occlusive atherosclerotic disease. In contrast, McGregor (1974b)compared the effects of sucrose, starch and glucose feeding in rats, on platelet ADP-induced and thrombin-induced aggregation and shape change. Increased rate of shape change with ADP was found in the rats given starch, but no other effects were found. Hornstra (1971) found more rapid thrombosis of arotic loops, as measured by the obliteration time, in rats given starch compared with those given sucrose.

Bridges (1965) showed that increased platelet stickiness in diabetics could be normalised by administering oral glucose. Also it was found that adding glucose, in vitro, caused increased platelet stickiness. Bennett (1967), in contrast, showed no effect on platelet adhesiveness after ingestion of glucose. However this discrepancy may be due to the use of different platelet adhesiveness measurement techniques. Bridges used the Wright's rotating bulb method (Wright 1941), where platelets are in contact with glass for 20 mins, and Bennett used Hellem's glass bead column technique (Hellem 1960), the contact time being only 26 seconds. Davis, Wilson and McField (1967) showed that, in vitro, addition of glucose to citrated platelet-rich plasma from diabetic and non-diabetic subjects, had no significant effect on ADP-induced platelet aggregation. However oral glucose significantly decreased aggregation in both diabetic and non-diabetic men, even after blood glucose had returned to normal. This suggested that some other factor was involved, possibly plasma non-esterified fatty acids known to fall after glucose ingestion.

CHAPTER 2

EXPERIMENTAL

i) Animals and diets

Female Wistar albino rats were used throughout. Animals were weighed and litter matched at weaning to form groups of ten animals, which were housed five animals to each cage and numbered by ear marking.

After weaning, the animals were fed for 25 d on stock diet in experiments described in Section A and for 10 d on stock diet for experiments described in other sections. This initial pre-experimental period was followed by a period of 21 d on experimental diet unless otherwise stated. Analysis was performed over 5 days; 2 animals from each group each day.

Animals were given food and tap water ad libitum in all experiments unless otherwise stated. Animals were weighed every 3-4 days throughout the experimental period.

The composition of the experimental diets is shown in Tables 1-12. The dry powdered constituents of the diet were mixed briefly, the oil added slowly, any supplements added and the diets then mixed for a further 1 hour. All diets were stored at $4^{o}C$.

The composition of experimental diets

Section A

Experiment 1

Table 1

Group No.	1 (g/kg)	2 (g/kg)
Casein	200	200
Maize Starch	400	370
Sucrose	150	150
Corn oil	100	100
Vitamin mixture*	10	10
Mineral mixture*	40	40
Solka floc	100	100
Oxonate	-	30

* See table 12.

Experiment 2

Table 2

Group No.	1 (g/kg)	2 (g/kg)
Fusarium Mould	400	400
Maize Starch	250	220
Sucrose	150	150
Vitamin mixture*	10	10
Mineral mixture*	40	40
Corn oil	100	100
Solka floc	50	50
Oxonate	-	30

* see table 12.

Experiment 3

Table 3

	g/kg
Casein	200
Maize Starch	550
Sucrose	50
Safflower oil	50
Vitamin mixture*	10
Mineral mixture*	40
Solka floc	100

* see table 12.

Experiment 4

Table 4

Group No.	1 g/kg	2 g/kg	3 g/kg	4 g/kg
Casein	-	50	125	200
Fusarium Mould	400	300	150	-
Maize Starch	220	257	307	370
Sucrose	150	150	150	150
Vitamin mixture*	10	10	10	10
Mineral mixture*	40	40	40	40
Corn oil	100	100	100	100
Solka floc	50	63	88	100
Oxonate	30	30	30	30

* see table 12.

Experiment 5 diet as for Experiment 2 (Table 2)

Experiment 6 diet as for Experiment 2 (Table 2)

Experiment 7

Table 5

Group No.	1 g/kg	2 g/kg	3 g/kg	4 g/kg
Casein	200	200	200	200
Maize Starch	200	170	200	170
Fructose	400	400	-	-
Glucose	-	-	400	400
Safflower oil	50	50	50	50
Vitamin mixture*	10	10	10	10
Mineral mixture*	40	40	40	40
Solka floc	100	100	100	100
Oxonate	-	30	-	30

* see table 12.

Section B

Experiment 1

Table 6

Group No.	1 g/kg	2 g/kg	3 g/kg	4 g/kg
Casein	200	130	90	50
Maize Starch	500	570	610	650
Sucrose	50	50	50	50
Corn oil	100	100	100	100
Vitamin mixture*	10	10	10	10
Mineral mixture*	40	40	40	40
Solka floc	100	100	100	100

* see table 12.

Experiment 2

Table 7

Group No.	1 g/kg	2 g/kg
Casein	200	50
Maize Starch	500	650
Sucrose	50	50
Safflower oil	100	100
Vitamin mixture*	10	10
Mineral mixture*	40	40
Solka floc	100	100

* see table 12.

Section C

Experiment 1

Table 8

Group No.	1 g/kg	2 g/kg	3 g/kg	4 g/kg
Casein	200	200	200	200
Maize Starch	550	400	550	400
Sucrose	50	50	50	50
Safflower oil	50	200	-	-
Coconut oil	-	-	50	200
Vitamin mixture*	10	10	10	10
Mineral mixture*	40	40	40	40
Solka floc	100	100	100	100

* see table 12.

Experiment 2

Table 9

Group No.	1 g/kg	2 g/kg
Casein	200	200
Maize Starch	550	200
Sucrose	50	400
Fat	50	50
Vitamin mixture*	10	10
Mineral mixture*	40	40
Solka floc	100	100

* see table 12.

Experiment 3

Table 10

Group No.	1 g/kg	2 g/kg	3 g/kg	4 g/kg
Casein	200	200	200	200
Maize Starch	200	550	200	550
Fructose	400	50	-	-
Glucose	-	-	400	50
Safflower oil	50	50	50	50
Vitamin mixture*	10	10	10	10
Mineral mixture*	40	40	40	40
Solka floc	100	100	100	100

* see table 12.

Section D

Table 11

Group No.	1 g/kg	2 g/kg	3 g/kg	4 g/kg	5 g/kg	6 g/kg	7 g/kg	8 g/kg
Casein	200	50	200	50	200	50	200	50
Maize Starch	50	200	200	350	400	550	550	700
Sucrose	400	400	400	400	50	50	50	50
Safflower oil	200	200	50	50	200	200	50	50
Vitamin mixture*	10	10	10	10	10	10	10	10
Mineral mixture*	40	40	40	40	40	40	40	40
Solka floc	100	100	100	100	100	100	100	100

* see table 12.

Table 12

Composition of Mineral and Vitamin mixtures

Mineral mixture

	g/kg		g/kg
KH_2PO_4	366.63	$MnSO_4.4H_2O$	5.01
$CaCO_3$	359.35	KI	0.75
NaCl	131.32	$CuSO_4.5H_2O$	0.45
$MgSO_4.7H_2O$	110.67	$ZnCO_3$	0.22
$FeSO_4.7H_2O$	25.41	$CoCl_2.6H_2O$	0.02

Dry constituents were finely powdered and mixed slowly for 2 hours.

Vitamin mixture

	g/kg		g/kg
p-amino benzoic acid	11.000	Menaphthone-K	0.200
Choline HCl	11.000	Riboflavin	0.193
Rovimix A + D	5.000	Thiamin HCl	0.058
Rovimix E	5.000	Folic acid	0.039
Inositol	3.900	Pyridoxine	0.039
Ca pantothenate	1.160	Biotin	0.004
Nicotinic acid	0.980	Vitamin B_{12}	0.001
		Maize Starch	961.430

Dry constituents were finely powdered. Vitamin B_{12} and Choline HCl were dissolved in 10 mls of absolute alcohol and mixed slowly with the dry constituents.

ii) Methods

Preparation of platelet-rich plasma (PRP)

Blood samples (2.0 ml) were collected by cardiac puncture from rats, under light ether anaesthesia, using 21 G syringe needles, into one-tenth volume of trisodium citrate (20 g/l). (See p 48A) The samples were mixed by gentle inversion and transferred to 5 ml plastic vials. A sample (0.1 ml) was taken for measurement of whole blood platelet count. The remaining 2.0 ml of blood was centrifuged for 7 secs at 3000 rpm, and then for 9 mins at 140 G in a bench MSE centrifuge. The supernatant PRP was carefully pipetted off using an Eppendorf pipette. Contact with glass was avoided at all times.

Platelet counts

Platelet counts were performed using a model ZB_1 Coulter Counter fitted with a 70 μ orifice tube. The best settings were found to be: Reciprocal of Amplitude $\frac{1}{4}$, Reciprocal of Aperture $\frac{1}{4}$, Aperture Matching Switch 20 KΩ and Gain Trim 3. The size of sample counted was set at 0.1 ml. The Upper Threshold was set at 50, and the Lower Threshold at 6. Isoton (Coulter Electronics Ltd.) was used as the diluting fluid dispensed from a Zipette to produce minimal agitation, and allowed to stand in the cuvettes overnight to minimize the background due to air bubbles and dust particles. Platelet counts obtained were corrected for coincidence using a 70 μ coincidence chart.

a) Whole blood platelet count

Citrated whole blood (0.1 ml) was diluted to 1 ml with Isoton containing 2.5 mg dipotassium ethylene diamine tetraacetic acid (K_2 EDTA) (50 μl of 40 g/l) per ml Isoton. Samples of this dilution (20 μl) were further diluted to 5 ml with Isoton. This dilution was centrifuged for 11 secs at 3000 rpm, and then for 40 secs at 520 G in a bench MSE centrifuge to sediment red blood cells. 3 ml of the supernatant was then diluted to 9 ml in Isoton and mixed by gentle inversion, producing a final dilution of 1:7,500. This final dilution was counted on the Coulter Counter.

b) Platelet-rich plasma (PRP) platelet count

Samples of PRP (20 µl) were added to 20 ml of Isoton containing 2 mg K_2EDTA. (1 ml of 40 g K_2EDTA/l in 19 ml Isoton). After mixing gently 1 ml of this dilution was added to 9 ml of Isoton giving a final dilution of 1:10,000. This was counted on the Coulter Counter.

Platelet aggregation studies

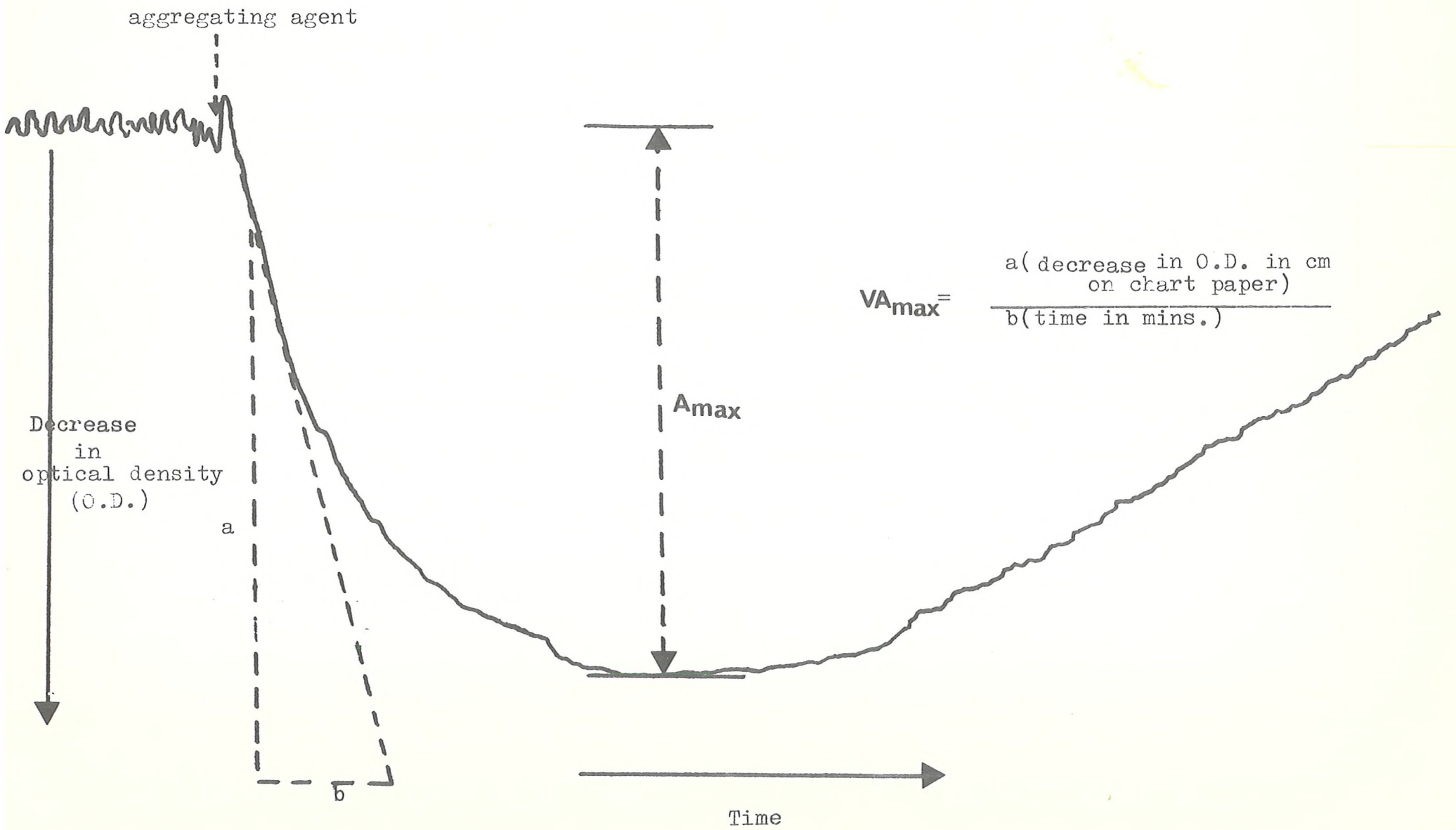
Aggregation was measured in an EEL 169 Aggregometer (Evans Electroselenium Ltd.) linked to a Servoscribe pen recorder, set at chart speed 30 cm/min, Sensitivity 5 mV and Zero 400%. Platelet-rich plasma was prepared and ADP-induced aggregation was measured 40 min after initial blood collection and thrombin-induced aggregation measured 50 min after initial collection. Samples of PRP (0.2 ml) were diluted to 1 ml with isotonic saline (pH 7.4) in a cuvette and placed in the aggregometer. The stirrer was set at speed 70 and, after allowing 2 min for the sample to reach 37°C, the aggregating agent was added using a Hamilton syringe. Using adenosine diphosphate (ADP) (Sigma) as the aggregating agent, 40 µl of 50 µM ADP was added, producing a final concentration of 2 µM ADP. Using thrombin (Diagnostic Reagents Ltd.) as the aggregating agent, 40 µl of 10 U thrombin/ml was added, producing a final concentration of 0.4 U thrombin/ml. Measurements taken from the aggregation trace recorded were the maximum rate of aggregation (VA max) and the maximum extent of aggregation (A max), and were corrected for PRP platelet count after performing the aggregation studies.

Kaolin-Cephalin clotting time

The Kaolin-Cephalin clotting time (KCCT) is a development of the partial thromboplastin time, the recalcification time and the whole blood clotting time, and measures deficiencies of factors I, II, V, VIII, IX, X, XI and XII (Biggs 1972).

Platelet-poor plasma was prepared by centrifuging at 1250 G for 15 min the blood remaining after the platelet-rich plasma had been removed. Samples of platelet-poor plasma (0.2 ml) were placed in the aggregometer cuvette and 0.4 ml of kaolin/platelet substitute added (Diagnostic Reagents Ltd.). The cuvette was immediately placed in the aggregometer and the stirrer set at the slowest speed (speed 30).

A typical example of an aggregation trace



$$VA_{max} = \frac{a(\text{decrease in O.D. in cm on chart paper})}{b(\text{time in mins.})}$$

At the end of exactly 2 min, 0.2 ml of 0.025 M calcium chloride was added and the 'start' switch on the aggregometer switched to 'read' and immediately back to 'start', which begins the timer. At the end of about 30 sec a rapid change in optical density occurred due to fibrin formation and deposition on the stirrer, resulting in the automatic timer cutting off. The Kaolin-Cephalin clotting time was taken as the time recorded on the timer.

Collection of samples for other analyses

After the initial collection of blood for aggregation studies, the rat thorax was opened to expose the heart from which a further blood sample (1 ml) was taken into one-tenth volume of trisodium citrate (31 g/l). This blood sample was centrifuged and the supernatant plasma stored in a deep freeze pending analysis.

The kidneys were excised and deep frozen for uric acid analysis.

Plasma uric acid

Frozen plasma samples were thawed and uric acid was determined using an Autoanalyzer II (Technicon Instruments Corporation 1971 Method sheet N-13a).

Kidney uric acid

Frozen kidneys were thawed, weighed and homogenised in 20 ml lithium carbonate (5 g/l) to solubilise uric acid deposits (Norrlind and Kihlberg 1972). The homogenates were centrifuged and filtered, and uric acid determined on the filtrate by the method used for plasma.

Kidney histology.

In some experiments kidney slices were taken immediately following excision, fixed in absolute alcohol and stored at $4^{\circ}C$. After embedding in wax sections were taken and stained for uric acid with Gomori's stain (Gomori 1951) with a background of Eosin and Haematoxylin.

Oxonate synthesis

Synthesis from uric acid was performed by the method of Brandenberger (1954), with slight modifications. During the purification steps, the product was washed with glass distilled water at 4°C, alcohol and diethyl ether. This produced a final yield of approximately 35%. The purity of the product was checked by comparing its spectrum at 200-300 nm with an oxonate spectrum published by Fridovich (1965).

Triglyceride and Cholesterol determinations

Frozen plasma samples were thawed and cholesterol and triglyceride concentrations were determined using an Autoanalyser II (Technicon methods sheet AA II-24).

Kidney protein determinations

Sections of kidney weighing about 100 mg were weighed accurately, homogenised in 5 ml trichloroacetic acid (50 g/l) and allowed to stand for 10 min. The samples were centrifuged and, after discarding the supernatant, the precipitated protein was washed twice with 1:1 Ether/Acetone mixture, being allowed to stand for 15 min before centrifuging each time. The precipitate was then dissolved in 1 ml of 0.4 M KOH in a 70°C water bath. Biuret reagent (6 ml) was added and after standing for 45 min the optical density was measured at 540 nm. This is a modification of the method of Lowry, Rosebrough, Farr and Randall (1951).

Statistical analysis of results

The Standard Error of the mean for each group of results was calculated from the formula:-

$$\text{Standard Error (SE)} = \sqrt{\frac{\sum(x - \bar{x})^2}{n(n - 1)}}$$

where x is the observation

$\bar{x}$ is the mean of the observations in that group

n is the number of observations in that group.

The statistical difference between two groups of observations (1 and 2) was evaluated by the Students 't' test, where:-

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{(SE_1)^2 + (SE_2)^2}}$$

The value of p, the probability that two groups of observations were statistically different, was obtained from the appropriate tables. Unless otherwise stated statistical differences were found to be non-significant.

Development of aggregation methods

Experiment to determine the effect of time after initial blood collection and of citrate concentration on ADP-induced aggregation uncorrected for PRP platelet count

The object of this experiment was to study the effect of using different concentrations of citrate as an anticoagulant on subsequent ADP-induced aggregation and to study the effect of allowing the platelet-rich plasma to stand at room temperature for different periods of time on subsequent aggregation. Whole blood samples were taken by cardiac puncture from stock 200 g male rats into one-tenth volumes of 15, 20, 25, 31 or 38 g trisodium citrate/l. Platelet-rich plasma was prepared from each blood sample and allowed to stand at room temperature for 20, 30, 40 or 50 min after initial collection and before aggregating with 2 μM ADP final concentration. The maximum rate of aggregation (VAmax) and the extent of aggregation (Amax) were measured. Results were obtained for 6 animals at each citrate concentration and are presented in the tables below and the figures on pages 48C and D.

The effect of time after initial blood collection and citrate concentration on the maximum rate of ADP-induced aggregation (VAmax) (cm/min)

Time (min) / Final citrate concentration (g/l)	20	30	40	50
3.8	1.24±0.19***	1.57±0.17**	2.22±0.20**	3.82±0.41
3.1	2.18±0.39***	3.13±0.46**	4.67±0.50	6.38±0.63
2.5	5.58±1.10**	7.00±1.03*	7.63±0.92	9.25±1.42
2.0	7.72±0.57***	9.77±0.74**	11.08±0.53**	12.15±0.69
1.5	10.32±1.08	11.22±0.82	11.77±0.93	11.92±0.91

Significance of difference from 50 min by paired Student 't' test - *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

The effect of time after initial blood collection and citrate concentration on the extent of ADP-induced aggregation (Amax)(cm)

Time (min) / Final citrate concentration (g/l)	20	30	40	50
3.8	1.15±0.18***	1.43±0.14***	2.20±0.22***	3.46±0.27
3.1	2.28±0.35***	3.00±0.41***	4.20±0.42**	5.60±0.33
2.5	5.00±0.81**	5.90±0.71**	6.78±0.64*	8.20±0.94
2.0	7.27±0.37***	8.92±0.57*	9.44±0.37*	10.03±0.44
1.5	8.80±0.86	9.88±0.89	10.32±0.72	10.63±0.71

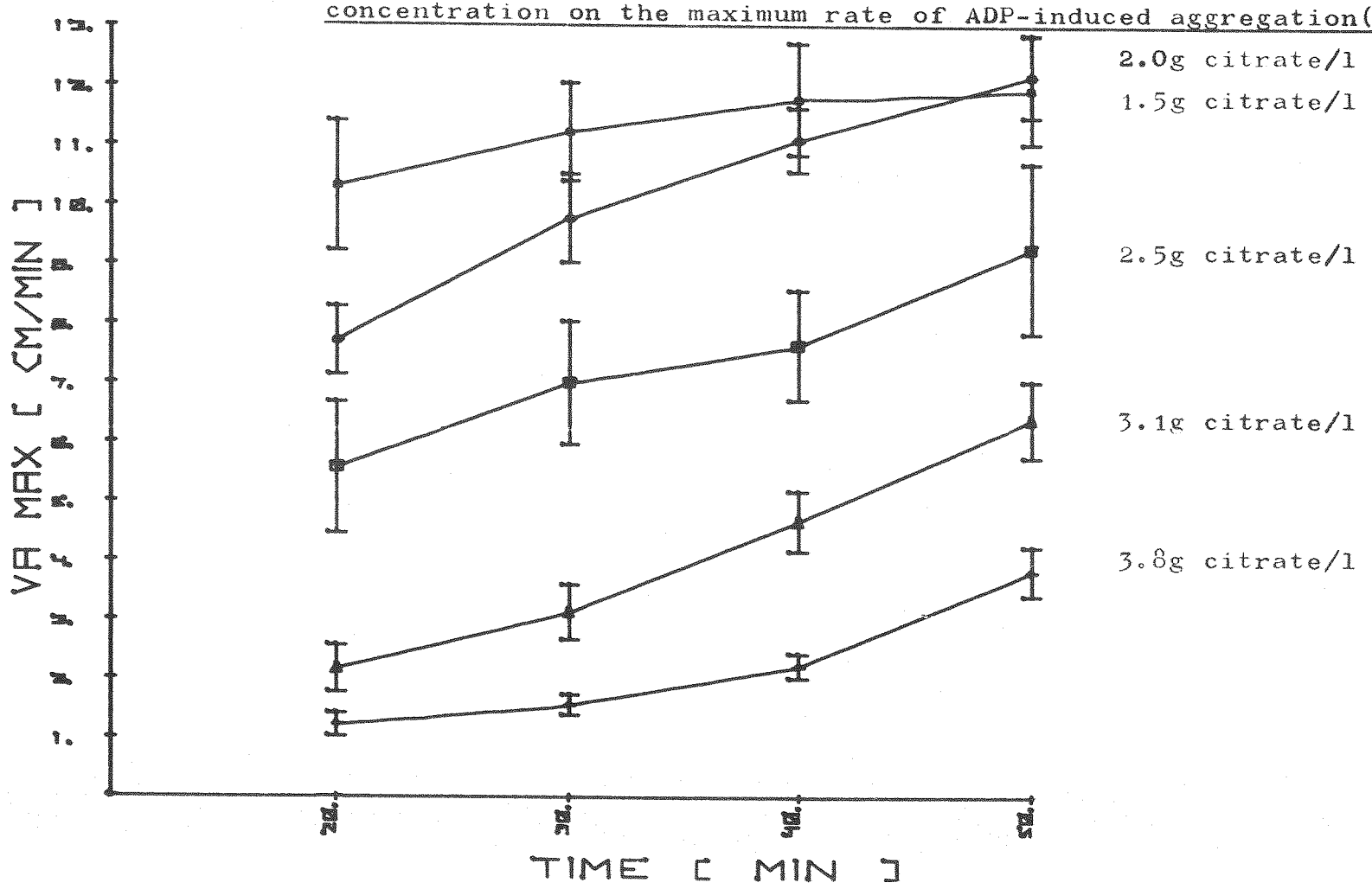
Significance of difference from 50 min by paired Student 't' test - *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

As the concentration of citrate was increased the rate and extent of aggregation decreased. The slopes of increasing rates and extents of aggregation with time were more marked at higher concentrations of citrate. At lower concentrations of citrate (2.0 and 1.5 g/l) the slopes of the curves were greatly reduced at 40 min. For this reason 40 min was the time chosen for ADP-induced aggregation. Thus thrombin-induced aggregation was able to be carried out subsequently at precisely 50 min after the initial collection of blood. The concentration of citrate chosen for aggregation studies was 2.0 g citrate/l since some coagulation was found to occur at 1.5gcitrate/l. This rather low concentration of citrate compared with more conventional concentrations of 3.1 or 3.8 g citrate/l, is however similar to that used by Macmillan and Sim (1970) (1.9 g citrate/l) in a comparative study of platelet aggregation in man and laboratory animals including the rat. In another comparative study (Sinakos and Caen 1967) the rat was found also to require a lower concentration of citrate than other mammalian species for the study of platelet aggregation.

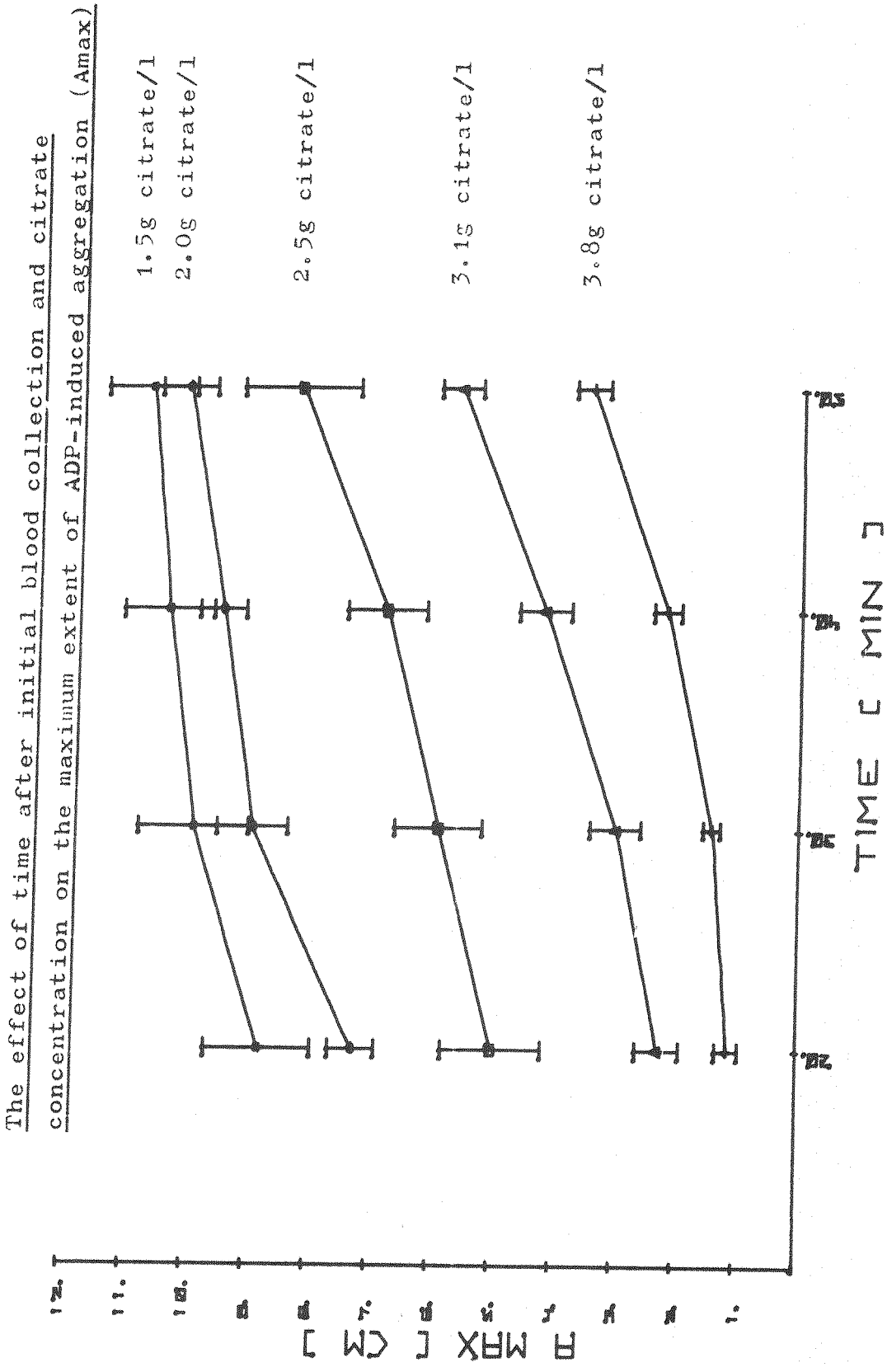
Correction in aggregation studies for platelet-rich plasma (PRP) platelet count.

Aggregation values were corrected for PRP platelet count after performing the aggregation studies, as described in the schedule for the EEL 169 aggregometer. This correction enabled direct comparisons of aggregation per 10^8 platelets to be made between experiments and between treatments within experiments even when whole blood platelet

The effect of time after initial blood collection and citrate concentration on the maximum rate of ADP-induced aggregation(VAmax)



The effect of time after initial blood collection and citrate concentration on the maximum extent of ADP-induced aggregation (Amax)



count was shown to be different. It was observed that the ratio platelet-rich plasma platelet count:whole blood platelet count was relatively constant for all dietary procedures within experiments.

Example of calculation

Dilution of PRP (0.2 ml) in saline (0.8 ml) = ×5

PRP platelet count = $x \times 10^8$ platelets/ml

∴ Diluted platelet count = $\frac{x \times 10^8}{5}$ platelets/ml

VAmax (uncorrected) = y cm/min

∴ VAmax corrected = $\frac{y}{x \times 10^8} \times 5 \times 10^8$ cm/min per 10^8 platelets

= $\frac{5y}{x}$ cm/min per 10^8 platelets

In some experiments the significance of the differences between treatments was increased by correcting to a standard platelet count, in others the differences were greater for the uncorrected counts but in no experiment did the correction procedure alter the direction of the observed treatment effects when significant differences were observed.

CHAPTER 3

RESULTS

Section A

Introduction

The increasing pressures of population and predicted increase in world food shortages have created a demand for new sources of human food. Research to develop feasible means of producing and utilizing, on a large scale, protein from single-cell organisms offers one of the best hopes for major new protein supplies independent of agricultural land use. Single-cell protein (SCP) is a source of protein derived from unicellular organisms such as bacteria, yeasts, fungi, algae and protozoa. The choice of a particular micro-organism depends upon a complicated balance between nutritional, engineering, food technological and economic factors, because a major objective is to produce not an ideal protein, but an adequate and cheap one. There has been some acceptance of the concept of micro-organisms for human food, when grown on various carbohydrates substrates, however there is, at present, a certain reluctance to sanction the use of SCP grown on petroleum fractions or derived hydrocarbons (Scrimshaw 1975). The major nutritional problem concerning the choice of SCP is not the nutritive value of the material, but its acceptability to the consumer. In human feeding trials, several SCP sources have led to a wide range of gastrointestinal symptoms from the relatively mild such as bulky stools and flatulence to the more serious such as nausea, vomiting and diarrhoea. It is unfortunate that some SCP material causes these adverse reactions in man, but not in laboratory animals (Calloway 1974).

One of the major limitations to the use of SCP as food for man is the high nucleic acid content of the cells. A high nucleic acid content is a characteristic of rapidly growing cells and micro-organisms contain between 8 and 25 grams nucleic acid (NA) per 100 g protein. The highest ribonucleic acid (RNA) content in animal tissue is found in intestinal mucosa, the pancreas, liver and kidneys. Liver contains about 4 g NA per 100 g protein, while some minor dietary components, such as sardines and fish roe contain 2.2 and 5.7 g NA per 100 g protein respectively (Miller 1968). Several methods have been developed that are designed to reduce nucleic acid content of SCP material. These methods include regulation of growth rate, chemical extraction processes and enzymic degradation of RNA. However

efficient extraction seems to require cell disintegration, which may be associated with loss of amino-acids and proteins. (Kihlberg 1972).

The final metabolic product formed in man from the purine moiety of the nucleic acids is uric acid (Figure 5,6). Uric acid is only slightly soluble at physiological pH's and, if the blood uric acid concentration is elevated, crystals may form in the joints as in the condition of gout. With excessive uric acid excretion, solubility of uric acid in the urine may be exceeded and stones may be deposited in the urinary tract. These problems do not exist in experimental animals, because in contrast to man, other higher apes and dalmation dogs, other mammals possess the enzyme uricase, which converts uric acid into the soluble and easily excretable metabolite allantoin (Figure 5,6). Because of the unique blockage in the purine metabolism in man, animal experiments for studies concerning dietary RNA effects are not directly applicable. However an animal model has been devised, for the study of the effects of feeding nucleic acid rich diets, involving the feeding of a specific uricase inhibitor, oxonate (Johnson et al 1969). In such a system changes in plasma uric acid concentration have been studied after the administration of RNA or SCP (Kihlberg 1972).

Expt. A1 The effects of feeding oxonate per se in the diet

Before considering the effects of feeding oxonate with nucleic acid-rich diets, it was essential to establish that oxonate had no effects per se on the variables it was intended to study.

Two groups of female rats were fed for 21 d on normal diets, with casein as the protein source: 30 g oxonate/kg diet was included in the experimental diet at the expense of maize starch (Table 1).

There was a 36% increase in the plasma uric acid concentration in the experimental, oxonate fed, group, compared to the control group (Table 14). The oxonate appeared therefore to be functional in its ability to inhibit the enzyme uricase, resulting in an increase in plasma uric acid concentration. Apart from this effect of oxonate, there were no other effects on any of the observations made, and therefore oxonate per se appears to have no detrimental effects on any of the variables it was intended to study (Tables 15,16).

Table 13

Expt. A1 The effect of feeding oxonate per se on body weight and energy intake

Addition to diet	Initial body wt. (g)	Final body wt. (g)	Energy intake (kJ/100 g.per day)
None (10)	176 ± 3	206 ± 5	128.0
Oxonate (10)	175 ± 4	195 ± 5	116.7

Results are the mean ± SEM for the number of observations in parenthesis.

Table 14

Expt. A1 The effect of feeding oxonate per se on plasma uric acid concentration, kaolin-cephalin clotting time and whole blood platelet count

Addition to diet	Plasma uric acid (mg/l)	Kaolin-cephalin clotting time (seconds)	Whole blood platelet count ($\times 10^8$/ml)
None	18.4 ± 1.9 (10)	36.0 ± 2.3 (7)	6.5 ± 0.2 (7)
Oxonate	25.1 ± 1.5* (10)	33.6 ± 1.6 (8)	6.7 ± 0.3 (8)

Results are the Mean ± SEM for the number of observations in parenthesis. Significance of difference from oxonate-free group - * $p < 0.02$

Table 15

Expt. A1 The effect of feeding oxonate per se on ADP-induced and thrombin-induced aggregation

Addition to diet	VAmax(cm/min per 10^8 platelets		Amax(cm/10^8 platelets)	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
None (7)	6.22±0.37	7.28±0.37	5.34±0.18	6.63±0.09
Oxonate (8)	5.45±0.40	6.64±0.33	5.06±0.40	6.18±0.33

Results are the Mean ± SEM for the number of observations in parenthesis

Table 16

Expt. A1 The effect of feeding oxonate per se on kidney weight and uric acid concentration

Addition to diet	Kidney wt. (g/100 g body wt.)	Kidney uric acid (mg/100 g. body wt.)	Kidney uric acid (mg/g tissue per 100 g body wt.)
None (10)	0.61 ± 0.02	0.19 ± 0.01	0.15 ± 0.01
Oxonate (10)	0.64 ± 0.01	0.20 ± 0.01	0.16 ± 0.01

Results are the Mean ± SEM for the number of observations in parenthesis.

Table 14A

Expt. A1 The effect of feeding oxonate per se on platelet-rich plasma platelet count (PRP) and on the ratio to whole blood platelet count (WB)

Addition to diet		Platelet-rich plasma platelet count ($\times 10^8$/ml)	Ratio PRP:WB
None	(7)	14.9 ± 0.7	2.3
Oxonate	(8)	14.7 ± 0.9	2.2

Results are the Mean ± SEM for the number of observations in parenthesis.

Table 15A

Expt. A1 The effect of feeding oxonate per se on ADP-induced and thrombin-induced aggregation (uncorrected)

Addition		VAmax (cm/min)		Amax(cm)	
to diet		ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
None	(7)	18.69±1.80	24.77±1.76	15.46±0.74	18.15±1.00
Oxonate	(8)	16.00±1.99	18.35±2.80	12.69±1.66	14.54±2.21

Results are the Mean ± SEM for the number of observations in parenthesis.

Expt. A2

The effect of feeding nucleic acid-rich diets (*Fusarium* mould) containing oxonate

Two groups of female rats were fed diets containing 400 g *Fusarium* mould/kg diet for 21 d prior to investigation: 30 g oxonate/kg diet was included in the experimental diet at the expense of maize starch (Table 2).

In the experimental, oxonate-fed, group the final body weight was reduced ($p < 0.001$) associated with a 26% reduction in energy intake. (Table 17). This was further investigated in Expt. A3.

There was an 83% increase in plasma uric acid concentration in the oxonate-fed group, reflecting the effectiveness of oxonate to inhibit uricase in animals given the high nucleic acid-rich diet. Kaolin -cephalin clotting time was increased ($p < 0.05$) and the whole blood platelet count was increased by 23% in the oxonate fed group (Table 18).

The maximum rate of aggregation (VAmax) for ADP-induced aggregation rose from 5.27 cm/min per 10^8 platelets in the control group to 7.09 cm/min per 10^8 platelets in the oxonate-fed group ($p < 0.01$). VAmax also rose for thrombin-induced aggregation from 6.19 cm/min per 10^8 platelets in the control group to 7.52 cm/min per 10^8 platelets in the oxonate-fed group ($p < 0.05$) (Table 19). There was also a small, non-significant, rise in the maximum extent of aggregation (Amax) for both ADP-induced and thrombin-induced aggregation.

Significant correlations between plasma uric acid concentrations and VAmax for ADP-induced ($p < 0.001$) and for thrombin-induced ($p < 0.01$) aggregation were demonstrated. Correlations between plasma uric acid concentration and Amax for ADP-induced ($p < 0.01$) and thrombin-induced ($p < 0.05$) aggregation were also significant (Table 20).

There was a 42% increase in kidney weight in the oxonate-fed group. Kidney protein and water concentration were, however, unchanged (Table 21). Kidney uric acid (mg/100 g body wt.) was increased 7 fold in the oxonate-fed group (Table 21), but this increase was insufficient to account for the increase in kidney weight.

In an attempt to identify the areas of the kidney, where the highest concentrations of uric acid occurred, histological studies were

Table 17

Expt. A2 The effect of nucleic-acid rich diets containing oxonate on body weight and energy intake

Addition to diet	Initial body wt. (g)	Final body wt. (g)	Energy intake (kJ/100 g per day)
None (10)	176 ± 4	208 ± 5	102.1
Oxonate (10)	171 ± 2	175 ± 4***	75.3

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from oxonate-free fed group - *** $p < 0.001$

Table 18

Expt. A2 The effect of nucleic-acid rich diets containing oxonate on plasma uric acid concentration, kaolin-cephalin clotting time and whole blood platelet count

Addition to diet	Plasma uric acid (mg/l)	Kaolin-cephalin clotting time (seconds)	Whole blood platelet count ($\times 10^8$/ml)
None	19.5 ± 1.7 (10)	34.3 ± 0.5 (7)	6.1 ± 0.1 (7)
Oxonate	35.6 ± 4.3** (9)	38.3 ± 1.5* (8)	7.5 ± 0.2*** (8)

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from oxonate-free fed group - * $p < 0.05$
** $p < 0.01$ *** $p < 0.001$

Table 19

Expt. A2 The effect of feeding nucleic acid-rich diets (Fusarium mould) containing oxonate on ADP-induced and thrombin-induced aggregation

Addition to diet	VAmax(cm/min per 10^8 platelets)		Amax(cm/10^8 platelets)	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
None (7)	5.27 ± 0.25	6.19 ± 0.35	4.84 ± 0.17	6.06 ± 0.19
Oxonate(8)	7.09 ± 0.56**	7.52 ± 0.42*	5.33 ± 0.58	6.67 ± 0.21

Results are the Mean ± SEM for the number of observations in parenthesis
Significance of difference from oxonate-free fed group = *p < 0.05
**p < 0.02

Table 20

Expt. A2 Relationship between platelet aggregation and plasma uric acid concentration

	Correlation coefficients (r)	
	VAmax	Amax
ADP-induced aggregation	0.88***	0.82**
Thrombin-induced aggregation	0.74**	0.65*

Significance of differences *p < 0.05 **p < 0.01 ***p < 0.001

Table 18A

Expt. A2 The effect of feeding nucleic acid-rich diets (Fusarium mould) containing oxonate on platelet-rich plasma platelet count (PRP) and on the ratio to whole blood platelet count (WB)

Addition to diet	Platelet rich plasma platelet count ($\times 10^8$/ml)	Ratio PRP:WB
None (7)	13.4 ± 0.4	2.2
Oxonate (8)	16.5 ± 0.6***	2.2

Results are the Mean ± SEM for the number of observations in parenthesis.

Significance of difference from oxonate-free fed group - *** $p < 0.001$

Table 19A

Expt. A2 The effect of feeding nucleic acid-rich diets (Fusarium mould) containing oxonate on ADP-induced and thrombin-induced aggregation (uncorrected)

Addition to diet	VAmax (cm/min)		Amax (cm)	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
None (7)	14.15±0.98	16.64±1.25	12.97±0.66	16.26±0.84
Oxonate (8)	23.34±1.80***	24.86±1.64**	16.32±1.15*	19.66±0.32*

Results are the Mean ± SEM for the number of observations in parenthesis

Significance of difference from oxonate-free fed group - * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Table 21

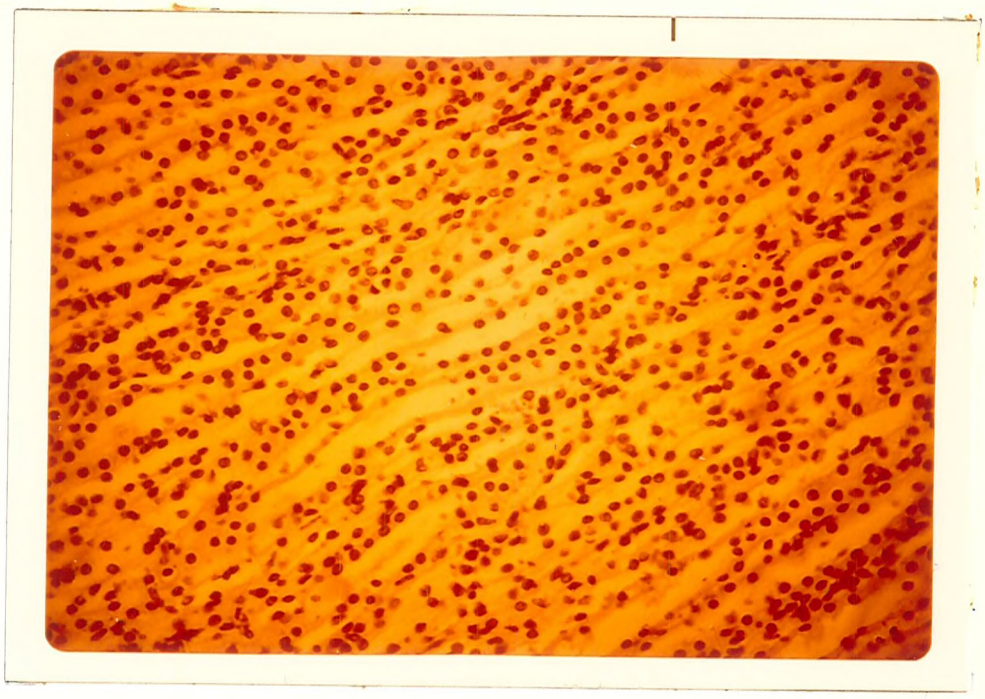
Expt. A2 The effect of feeding nucleic acid-rich diets (*Fusarium* mould) containing oxonate on kidney weight, protein, water and uric acid concentation

Addition to diet	Kidney wt. (g/100 g body wt)	Kidney protein (mg/g tissue)	Kidney water (%)	Kidney uric acid (mg/100 g body wt.)	Kidney uric acid (mg/g tissue per 100 g body wt.)
None	0.67 ± 0.02 (10)	120 ± 7 (4)	79.3 ± 0.7 (4)	0.22 ± 0.01 (10)	0.16 ± 0.01 (10)
Oxonate	0.95 ± 0.02*** (10)	117 ± 5 (4)	79.5 ± 0.6 (4)	1.55 ± 0.22*** (10)	0.92 ± 0.12*** (10)

Results are the Mean ± SEM for the number of observations in parenthesis.

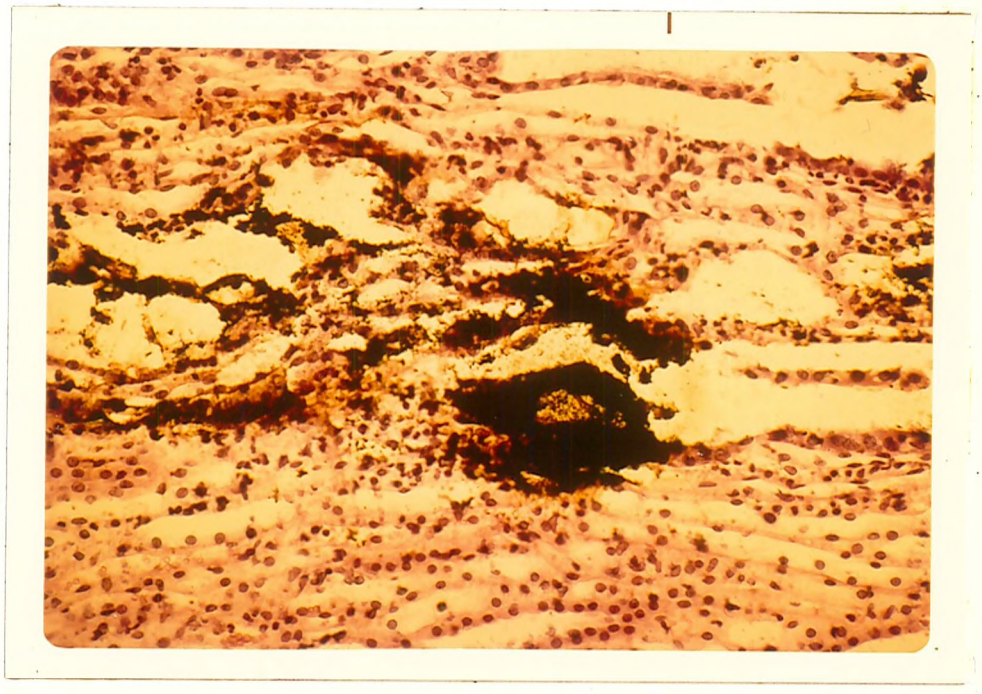
Significance of difference from oxonate-free fed group *** $p < 0.001$

Plate 1 - Control L.S.



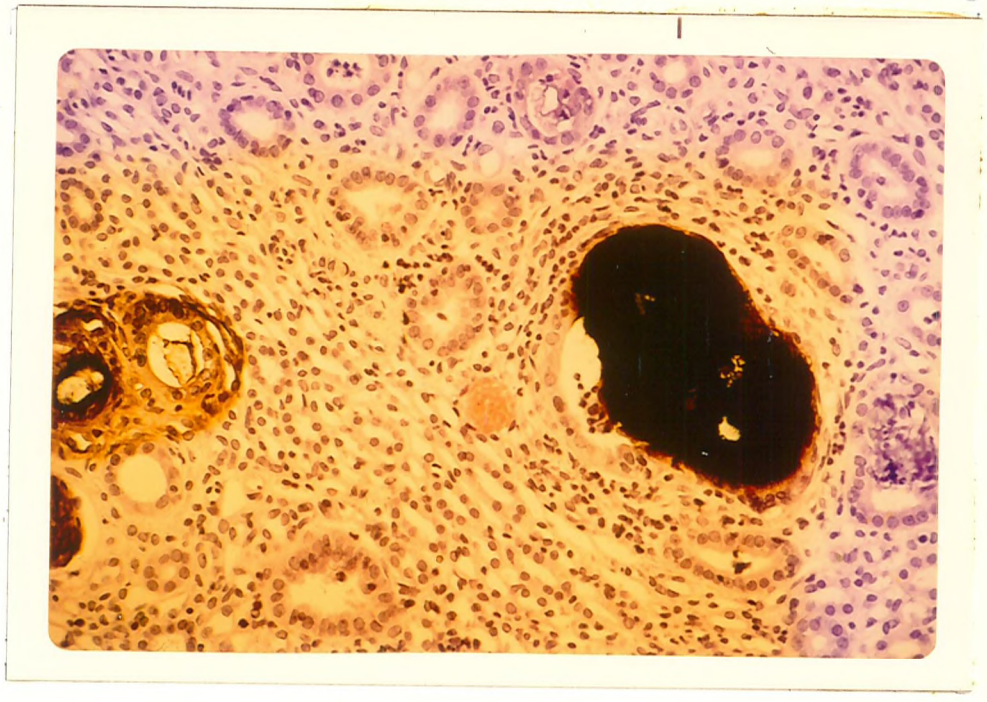
Magnification X 350

Plate 2 - Experimental L.S.



Magnification X 400

Plate 3 - Experimental T.S.



Magnification X 400

Note Background colour on control slide is slightly different to experimental slides due to the control and experimental kidney sections being photographed on different occasions, but staining procedures for all sections was identical.

performed. Heavy black deposits of uric acid were seen confined to the tubules of the pyramid region and less severe brown deposits were found in the tissue surrounding the tubules (Plates 2,3). Plate 1 shows a kidney taken from a rat fed the control diet, which had undergone identical staining procedure.

Expt. A3

The effects of food restriction

There was a reduced food intake in rats fed nucleic acid-rich diets containing oxonate compared to the oxonate-free control animals (Expt. A2). The effect of food restriction per se on platelet aggregation was therefore studied.

Two groups of individually caged female rats were fed 200 g casein per kg diet (Table 3) for 21 d prior to investigation. The control group was fed ad libitum, while the experimental group was restricted to a daily intake of 9 g/rat per day.

The final body weight was reduced in the food restricted group ($p < 0.001$) (Table 22).

There was a reduction in VAmax in the food restricted group for both ADP-induced ($p < 0.01$) and thrombin-induced ($p < 0.001$) aggregation. Similarly Amax was reduced for both ADP-induced ($p < 0.01$) and thrombin-induced ($p < 0.05$) aggregation in the experimental group (Table 23).

The increase in aggregation tendency in animals fed nucleic acid-rich diets containing oxonate, observed in Expt. A2, cannot therefore be explained by the reduced food intake of the group, since reducing food intake per se decreases the tendency of platelets to aggregate.

Expt. A4

The effect of increasing the proportion of mould in oxonate-supplemented diets

Feeding rats on nucleic acid-rich diets (400 g Fusarium mould/kg diet) containing oxonate led to an increase in ADP-induced and thrombin-induced aggregation (Expt. A2). The proportion of mould in oxonate-supplemented diets needed for this effect to occur was therefore investigated.

Table 22

Expt. A3. The effect of food restriction on body weight, energy intake kaolin-cephalin clotting time and whole blood platelet count

	Initial body wt. (g)	Final body wt. (g)	Energy intake (kJ/100 g per day)	Kaolin-cephalin clotting time (seconds)	Whole blood platelet count ($\times 10^8$/ml)
Control	136 ± 6 (20)	186 ± 4 (20)	156.7 (20)	34.5 ± 1.6 (16)	6.2 ± 0.3 (16)
Restricted†	137 ± 7 (20)	129 ± 4*** (20)	107.3 (20)	30.0 ± 1.7 (10)	6.2 ± 0.2 (10)

† Food intake - 9 g/rat per day

Results are the Mean ± SEM for the number of observations in parenthesis.

Significance of difference from Control group - *** $p < 0.001$.

Table 23

Expt. A3 The effect of food restriction on ADP-induced and thrombin-induced aggregation

	VAmax(cm/min per 10^8 platelets)		Amax(cm/10^8 platelets)	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
Control (16)	4.14 ± 0.25	5.40 ± 0.23	3.88±0.18	4.63 ± 0.20
Restricted†(10)	2.98 ± 0.19**	4.21 ± 0.16***	3.02±0.14**	4.00 ± 0.16*

† Food Intake - 9 g/rat per day.

Results are the Mean ± SEM for the number of observations in parenthesis.

Significance of difference from Control group - *$p < 0.05$ **$p < 0.01$ *** $p < 0.001$

Table 22A

Expt A3 The effect of food restriction on platelet-rich plasma platelet count (PRP) and on the ratio to whole blood platelet count (WB)

		Platelet-rich plasma platelet count ($\times 10^8$/ml)	Ratio PRP:WB
Control	(16)	12.5 ± 0.6	2.0
Restricted†	(10)	12.2 ± 0.4	2.0

† Food intake - 9g/rat per day

Results are the Mean ± SEM for the number of observations in parenthesis.

Table 23A

Expt. A3 The effect of food restriction on ADP-induced and thrombin-induced aggregation (uncorrected)

		VAmax (cm/min)		Amax (cm)	
		ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
Control	(16)	10.64±1.00	13.76±1.09	9.81±0.73	11.73±0.84
Restricted†	(10)	7.29±0.62**	10.22±0.52**	7.41±0.59*	9.70±0.49

† Food intake - 9 g/rat per day

Results are the Mean ± SEM for the number of observations in parenthesis.

Significance of difference from Control group - * $p < 0.05$ ** $p < 0.02$

Four groups of female rats were fed for 21 d prior to investigation , on diets containing 0, 150, 300 or 400 g Fusarium mould/kg diet, the protein content of the diets being balanced with casein. All diets also contained 30 g oxonate/kg diet (Table 4).

There was a progressive increase in plasma uric acid concentration with increasing levels of mould in the diet. The whole blood platelet count was elevated ($p < 0.01$), when the level of mould in the diet was 400 g kg diet (Table 25).

The maximum rate of aggregation (VAmax) for both ADP-induced and thrombin-induced aggregation increased progressively with increasing levels of mould in the diet (Table 26, Figures 9 and 10). There were slight, but less marked, rises in the maximum extent of aggregation (Amax) with increasing levels of mould.

Kidney weight increased by 29% when animals were fed 300 g mould per kg diet and when 400 g mould per kg diet was fed the increase in kidney weight was 35%. There was a 2 fold rise in the concentration of uric acid in the kidney (mg/100 g body wt.) of rats given 300 g mould/kg diet and rose to a 5 fold increase when rats were fed 400 g mould/kg diet (Table 27).

Expt. A5

The effect of the time during which oxonate is included in nucleic acid-rich diets

Feeding rats on nucleic acid-rich diets containing oxonate for 21 d led to an increase in ADP-induced and thrombin-induced aggregation (Expt. A2). The time-course of this effect on aggregation tendency was therefore studied.

Four groups of female rats were fed on diets containing 400 g Fusarium mould and 30 g oxonate per kg diet (Table 2) for 0, 4, 14 or 21 d prior to investigation. Animals were fed in such a way that all animals finished their period on experimental diet at the same time. The 0 d control group and other groups, when not on experimental diet, were fed diets containing 400 g mould per kg diet, but no oxonate.

There was a reduced final body weight in all groups compared to the 0 d control group (4 d $p < 0.05$; 14 d $p < 0.001$; 21 d $p < 0.001$) (Table 28) resulting from a reduced food intake as found in previous experiments.

Table 24

Expt. A4 The effect of increasing the proportion of mould in oxonate-supplemented diets on body weight and energy intake

Mould addition to diet (g/kg)	Initial body wt. (g)	Final body wt. (g)	Energy intake (kJ/100 g per day)
0 (10)	152 ± 3	181 ± 4	122.7
150 (10)	157 ± 2	189 ± 3	116.1
300 (10)	152 ± 2	179 ± 4	110.4
400 (40)	160 ± 2	178 ± 3	90.3

Results are the Mean ± SEM for the number of observations in parenthesis

Table 25

Expt. A4 The effect of increasing the proportion of mould in oxonate-supplemented diets on plasma uric acid concentration, kaolin-cephalin clotting time and whole blood platelet count

Mould addition to diet (g/kg)	Plasma uric acid (mg/l)	Kaolin-cephalin clotting time (seconds)	Whole blood platelet count ($\times 10^8$/ml)
0	27.4 ± 1.4 (10)	32.0 ± 1.6 (5)	5.9 ± 0.1 (5)
150	37.2 ± 2.4** (10)	31.9 ± 1.1 (8)	6.4 ± 0.2 (8)
300	39.8 ± 4.9* (10)	32.1 ± 1.0 (8)	6.5 ± 0.3 (8)
400	44.0 ± 2.4*** (39)	32.7 ± 1.0 (30)	7.0 ± 0.2** (30)

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from 0 addition - *$p < 0.05$ **$p < 0.01$
***$p < 0.001$

Table 26

Expt. A4 The effect of increasing the proportion of mould in oxonate-supplemented diets on ADP-induced and thrombin-induced aggregation

Mould addition to diet (g/kg)	VAmax(cm/min per 10^8 platelets)		Amax(cm/10^8 platelets)	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
0 (5)	3.99 ± 0.25	4.68 ± 0.35	3.96 ± 0.22	4.92 ± 0.20
150 (8)	4.37 ± 0.16	5.22 ± 0.17	4.38 ± 0.16	5.30 ± 0.06
300 (8)	4.72 ± 0.20*	5.58 ± 0.19*	4.71 ± 0.15*	5.28 ± 0.13
400 (30)	5.49 ± 0.27*	6.31 ± 0.22**	4.58 ± 0.16	5.42 ± 0.17

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from 0 addition group - *p < 0.05 ** p < 0.01

Table 27

Expt. A4 The effect of increasing the proportion of mould in oxonate-supplemented diets on kidney weight and uric acid concentration

Mould addition to diet (g/kg)	Kidney wt. (g/100 g body wt.)	Kidney uric acid (mg/100 g body wt.)	Kidney uric acid (mg/g tissue per 100 g body wt.)
0 (10)	0.75 ± 0.03	0.28 ± 0.02	0.20 ± 0.01
150 (10)	0.77 ± 0.03	0.28 ± 0.02	0.20 ± 0.01
300 (10)	0.97 ± 0.04***	0.60 ± 0.08***	0.34 ± 0.04**
400 (40)	1.01 ± 0.03***	1.43 ± 0.12***	0.79 ± 0.06***

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from 0 addition group - **p < 0.01 ***p < 0.001.

Table 25A

Expt. A4 The effect of increasing the proportion of mould in oxonate-supplemented diets on platelet-rich plasma platelet count (PRP) and on the ratio to whole blood platelet count (WB)

Mould addition to diet (g/kg)	Platelet-rich plasma platelet count ($\times 10^8$/ml)	Ratio PRP:WB
0 (5)	12.3 ± 0.3	2.1
150 (8)	12.3 ± 0.5	1.9
300 (8)	12.1 ± 0.5	1.9
400 (30)	14.4 ± 0.4*	2.1

Results are the Mean ± SEM for the number of observations in parenthesis
Significance of difference from 0 addition - *$p < 0.05$

Table 26A

Expt. A4 The effect of increasing the proportion of mould in oxonate-supplemented diets on ADP-induced and thrombin-induced aggregation (uncorrected)

Mould addition to diet (g/kg)	VAmax (cm/min)		Amax (cm)	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
0 (5)	9.82±0.70	11.46±0.78	9.77±0.72	12.11±0.57
150 (8)	10.77±0.74	12.80±0.74	10.81±0.74	12.99±0.60
300 (8)	11.48±0.88	13.53±0.87	11.41±0.67	12.79±0.75
400 (30)	16.14±1.06*	18.51±1.03**	12.86±0.58*	14.98±0.69

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from 0 addition - *$p < 0.05$ **$p < 0.01$

Figure 9 The effect of increasing the proportion of mould in oxonate-supplemented diets on ADP-induced aggregation

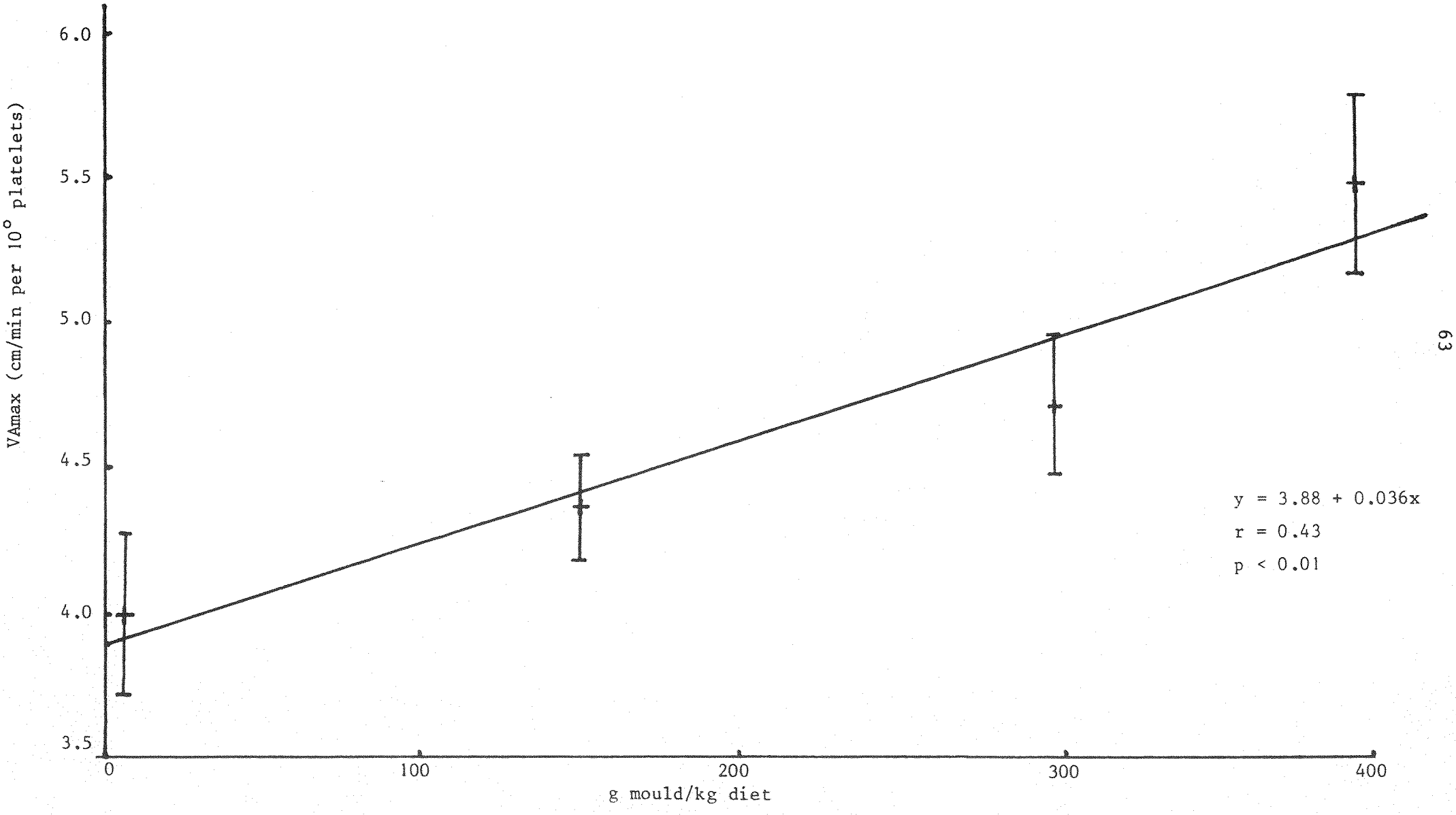
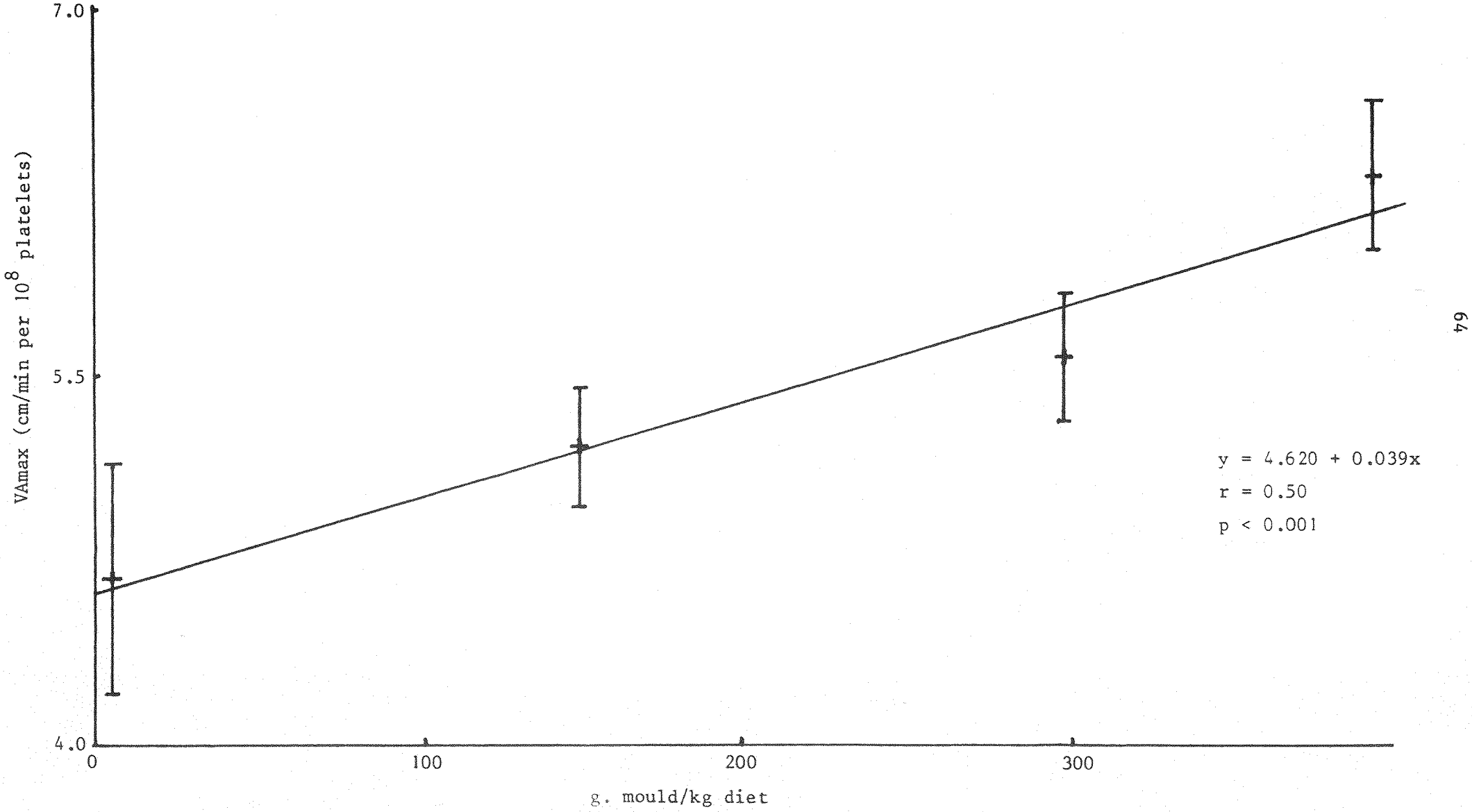


Figure 10 The effect of increasing the proportion of mould in oxonate-supplemented diets on thrombin-induced aggregation



Plasma uric acid concentration increased progressively with increasing time on diets containing oxonate (Table 29). Kaolin-cephalin clotting time was increased in the 21 d fed group ($p < 0.05$). Whole blood platelet count was increased by 12% in the 4 d fed group and became further increased with longer durations on the experimental diet (14 d 14% increase; 21 d 19% increase).

The maximum rate of aggregation (VAmax) for both ADP-induced and thrombin-induced aggregation did not increase until after 21 d on experimental diet (ADP-induced $p < 0.01$; thrombin-induced $p < 0.02$) (Table 30). Nor was the maximum extent of aggregation (Amax) for ADP-induced aggregation increased until after 21 d on experimental diet ($p < 0.05$). There appears therefore to be a time lag between the increase in plasma uric acid concentration and the increase in aggregation tendency.

Kidney weight had increased by 28% in the 14 d group and was increased by 33% in the 21 d group (Table 31). Kidney uric acid concentration (mg/100 g body wt.) increased progressively with time on oxonate diet and was increased 4 fold after 21 d on this diet.

Expt. A6

The effect of withdrawing oxonate from the diet after an initial 21 d feeding period

A time lag existed between the increase in plasma uric acid concentration and the increase in aggregation tendency (Expt. A5). This time lag was further investigated by studying the decay in increased aggregation tendency, resulting from feeding nucleic acid-rich diets containing oxonate for 21 d, when oxonate is withdrawn from the diet.

Five groups of female rats were fed diets containing 400 g Fusarium mould per kg diet, the experimental diets also including 30 g oxonate per kg diet (Table 2). The control group was fed the diet unsupplemented with oxonate for 21 d prior to investigation and the other four experimental groups were fed diets with added oxonate for 21 d followed by periods of 0, 5, 10 or 22 d on control, oxonate-free diets prior to analysis.

Plasma uric acid concentration had increased ($p < 0.001$) after 21 d on oxonate diet compared to the oxonate-free control fed group,

Table 28

Expt. A5 The effect of the time during which oxonate is included in nucleic acid-rich diets on body weight and energy intake

Oxonate in diet for (d)	Initial body wt. (g)	Final body wt. (g)	Energy intake (kJ/100 g per day)
0 (30)	162 ± 3	204 ± 3	110.5
4 (20)	155 ± 2	194 ± 3*	78.6
14 (20)	156 ± 2	183 ± 3***	85.4
21 (40)	160 ± 2	178 ± 3***	90.3

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from 0 d ; *p < 0.05 ***p < 0.001

Table 29

Expt. A5 The effect of the time during which oxonate is included in nucleic acid-rich diets on plasma uric acid concentration, kaolin-cephalin clotting time and whole blood platelet count

Oxonate in diet for (d)	Plasma uric acid (mg/l)	Kaolin-cephalin clotting time (seconds)	Whole blood platelet count (× 10^8/ml)
0	19.3 ±1.1 (29)	29.5 ± 0.9 (22)	5.9 ± 0.2 (22)
4	34.6 ±3.2*** (20)	31.2 ± 1.1 (16)	6.6 ± 0.2* (16)
14	39.2 ±3.8*** (20)	31.6 ± 0.6 (15)	6.7 ± 0.2** (15)
21	44.0 ±2.4*** (38)	32.7 ± 1.0* (30)	7.0 ± 0.2*** (30)

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from 0 d - *p < 0.05 **p < 0.01 ***p < 0.001

Table 30

Expt. A5 The effect of the time during which oxonate is included in nucleic acid-rich diets on ADP-induced and thrombin-induced aggregation

Oxonate in diet for (d)	VAmax(cm/min per 10^8 platelets)		(Amax(cm/10^8 platelets)	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
0 (22)	4.51±0.19	5.58 ± 0.19	4.11 ± 0.16	5.09 ± 0.20
4 (16)	4.40±0.12	5.63 ± 0.20	4.25 ± 0.14	5.24 ± 0.13
14 (15)	5.02±0.24	6.06 ± 0.21	4.47 ± 0.14	5.19 ± 0.13
21 (30)	5.49±0.27***	6.31 ± 0.22**	4.58 ± 0.16*	5.42 ± 0.17

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from 0 d - *p < 0.05 **p < 0.02 ***p < 0.01

Table 31

Expt. A5 The effect of the time during which oxonate is included in nucleic acid-rich diets on kidney weight and uric acid concentration

Oxonate in diet for (d)	Kidney wt. (g/100 g body wt.)	Kidney uric acid (mg/100 g body wt.)	Kidney uric acid (mg/g tissue per 100 g body wt.)
0 (30)	0.76 ± 0.02	0.36 ± 0.02	0.24 ± 0.01
4 (20)	0.80 ± 0.03	0.52 ± 0.03***	0.34 ± 0.03***
14 (20)	0.97 ± 0.04***	0.58 ± 0.08**	0.34 ± 0.04*
21 (40)	1.01 ± 0.03***	1.43 ± 0.12***	0.79 ± 0.06***

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from 0 d - *p < 0.02 **p < 0.01 ***p < 0.001

Table 29A

Expt. A5 The effect of the time during which oxonate is included in nucleic acid-rich diets on platelet-rich plasma platelet count (PRP) and on the ratio to whole blood platelet count (WB)

Oxonate in diet for (d)	Platelet-rich plasma platelet count ($\times 10^8$/ml)	Ratio PRP:WB
0 (22)	12.3 ± 0.5	2.1
4 (16)	13.1 ± 0.5	2.0
14 (15)	13.1 ± 0.5	2.0
21 (30)	14.4 ± 0.4**	2.1

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from 0 d - ** $p < 0.01$

Table 30A

Expt. A5 The effect of the time during which oxonate is included in nucleic acid-rich diets on ADP-induced and thrombin-induced aggregation (uncorrected)

Oxonate in diet for (d)	VAmax (cm/min)		Amax(cm)	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
0 (22)	11.30±0.87	13.91±0.99	10.32±0.78	12.45±0.92
4 (16)	11.57±0.65	14.80±0.84	11.17±0.65	13.69±0.58
14 (15)	13.26±0.87	15.94±0.88	11.75±0.61	13.61±0.59
21 (30)	16.14±1.06**	18.52±1.03**	12.86±0.58**	14.98±0.69*

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from 0 d - * $p < 0.05$ ** $p < 0.01$

and had fallen to its original concentration after 5 d withdrawal of oxonate from the diet (Table 33). Whole blood platelet was increased ($p < 0.001$) after 21 d on oxonate diet, and had fallen slightly, though non-significantly, after 22 d on oxonate-free diet.

The maximum rate of aggregation (VAmax) , which was elevated after 21 d on the experimental diet, had fallen from this elevated level after the 10 d oxonate-free period for both ADP-induced ($p < 0.05$) and thrombin-induced ($p < 0.01$) aggregation (Table 34). There appeared to be a time-lag between the fall in plasma uric acid concentration, which occurred after 5 d oxonate-free diet, and the fall in aggregation tendency, which occurred after 10 d oxonate-free diet.

The elevated kidney weight and uric acid concentration, which occurred after 21 d on the oxonate diet, had fallen, when oxonate was withdrawn from the diet for 5 d (kidney uric acid $p < 0.001$) and fell further after 10 d (kidney weight $p < 0.001$; kidney uric acid $p < 0.001$) (Table 35).

Expt. A7

The effect of feeding oxonate-supplemented diets rich in fructose or glucose

It has been reported that administration of fructose either by single oral dose or intravenously resulted in elevated plasma uric acid concentrations (Emmerson 1974; Perteentupa and Raivio 1967; Stirpe et al 1970; Heuchenkamp et al 1971; Kogut et al 1975). An experiment was therefore performed to investigate if hyperuricaemia could be induced in rats by chronic fructose feeding with or without oxonate and, if so, what the effect on platelet aggregation might be, in view of the demonstrated increased aggregation tendency in animals made hyperuricaemic by feeding nucleic acid-rich diets containing oxonate (Expt. A2).

Four groups of female rats were fed diets containing 400 g fructose or 400 g glucose/kg diet with or without 30 g oxonate/kg diet (Table 5) for 21 d prior to analysis.

Plasma uric acid concentration was increased in the fructose plus oxonate ($p < 0.05$) and the glucose plus oxonate ($p < 0.01$) fed groups compared to the oxonate-free control groups, but there was no

Table 32

Expt. A6 The effect of withdrawing oxonate from the diet after an initial 21 d feeding period on body weight

		Initial body wt. (g)	Final body wt. (g)
21 d mould	(30)	162 ± 3	204 ± 3
21 d mould + oxonate	(40)	160 ± 2	178 ± 3
plus 5 d oxonate free	(10)	149 ± 2	183 ± 3
plus 10 d oxonate free	(20)	159 ± 4	205 ± 5
plus 22 d oxonate free	(10)	167 ± 4	223 ± 5

Results are the Mean ± SEM for the number of observations in parenthesis.

Table 33

Expt. A6 The effect of withdrawing oxonate from the diet after an initial 21 d feeding period on plasma uric acid concentration, kaolin-cephalin clotting time and whole blood platelet count

	Plasma uric acid (mg/l)	Kaolin-cephalin clotting time (seconds)	Whole blood platelet count (× 10 /ml)
21 d mould	19.3±1.1*** (29)	29.5 ± 0.9* (22)	5.9 ± 0.2*** (22)
21 d mould + oxonate	44.0±2.4 (38)	32.7 ± 1.0 (30)	7.0 ± 0.2 (30)
plus 5 d oxonate free	21.2±1.5*** (9)	29.1 ± 1.5 (7)	6.7 ± 0.4 (7)
plus 10 d oxonate free	25.2±1.0*** (20)	32.3 ± 1.0 (13)	6.8 ± 0.2 (13)
plus 22 d oxonate free	21.6±2.5*** (10)	38.1 ± 2.0* (7)	6.5 ± 0.2 (7)

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from '21 d mould + oxonate' - *$p < 0.05$
***$p < 0.001$.

Table 34

Expt. A6 The effect of withdrawing oxonate from the diet after an initial 21 d feeding period on ADP-induced and thrombin-induced aggregation

		VAmax (cm/min per 10^8 platelets)		Amax (cm/10^8 platelets)	
		ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
21 d mould	(22)	4.51 ± 0.19***	5.58 ± 0.19*	4.11 ± 0.16*	5.09 ± 0.20
21 d mould + oxonate	(30)	5.49 ± 0.27	6.31 ± 0.22	4.58 ± 0.16	5.42 ± 0.17
plus 5 d oxonate free	(7)	5.06 ± 0.32	6.28 ± 0.42	4.52 ± 0.18	5.18 ± 0.28
plus 10 d oxonate free	(13)	4.35 ± 0.21*	4.98 ± 0.31**	4.57 ± 0.15	5.11 ± 0.16
plus 22 d oxonate free	(7)	4.30 ± 0.26*	4.80 ± 0.31**	4.32 ± 0.20	4.92 ± 0.35

Results are the Mean ± SEM for the number of observations in parentheses.

Significance of difference from '21 d mould + oxonate' - *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

Table 33A

Expt. A6 The effect of withdrawing oxonate from the diet after an initial 21 d feeding period on platelet-rich plasma platelet count (PRP) and on the ratio to whole blood platelet count (WB)

		Platelet-rich plasma platelet count ($\times 10^8$/ml)	Ratio PRP:WB
21 d mould	(22)	12.3 ± 0.6**	2.1
21 d mould + oxonate	(30)	14.4 ± 0.4	2.1
plus 5d oxonate free	(7)	12.4 ± 0.4*	1.9
plus 10d oxonate free	(13)	13.0 ± 0.4*	1.9
plus 22d oxonate free	(7)	12.9 ± 0.5	2.0

Results are the Mean ± SEM for the number of observations in parenthesis
Significance of difference from '21 d mould + oxonate' - *p < 0.05
**p < 0.01

Table 34A

Expt. A6 The effect of withdrawing oxonate from the diet after an initial 21 d feeding period on ADP-induced and thrombin-induced aggregation (uncorrected)

		VAmax (cm/min)		Amax (cm)	
		ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
21 d mould	(22)	11.30±0.87**	13.91±0.99**	10.32±0.78**	12.45±0.92*
21 d mould + oxonate	(30)	16.14±1.06	18.52±1.03	12.86±0.58	14.98±0.69
plus 5d oxonate free	(7)	12.64±1.10	15.69±1.45	11.23±0.71	12.87±0.95
plus 10d oxonate free	(13)	11.27±0.65**	12.92±0.90**	11.83±0.50	13.26±0.61
plus 22d oxonate free	(7)	11.21±1.04*	12.34±0.91**	11.15±0.75	12.76±1.24

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from '21 d mould + oxonate' - *p < 0.05
**p < 0.01

Table 35

Expt. A6 The effect of withdrawing oxonate from the diet after an initial 21 d feeding period on kidney weight and uric acid concentration

		Kidney wt. (g/100 g body wt.)	Kidney uric acid (mg/100 g body wt.)	Kidney uric acid (mg/g tissue per 100 g body wt.)
21 d mould	(30)	0.76 ± 0.02***	0.36 ± 0.02***	0.24 ± 0.01***
21 d mould + oxonate	(40)	1.01 ± 0.03	1.43 ± 0.12	0.79 ± 0.06
plus 5 d oxonate free	(10)	0.95 ± 0.03	0.53 ± 0.02***	0.31 ± 0.01***
plus 10 d oxonate free	(20)	0.79 ± 0.02***	0.45 ± 0.01***	0.28 ± 0.01***
plus 22 d oxonate free	(10)	0.83 ± 0.05**	0.41 ± 0.03***	0.23 ± 0.02***

Results are the Mean ± SEM for the number of observations in parenthesis.

Significance of difference from '21 d mould + oxonate' - **p < 0.01 ***p < 0.001

difference between the oxonate fed groups (Table 37). Whole blood platelet count was increased in the high glucose plus oxonate fed group ($p < 0.05$) compared to its oxonate-free control group, and the same trend, although non-significant, existed in the fructose fed groups.

No significant differences were observed in any of the aggregation studies performed (Table 38).

Kidney weight was raised by 16% in the fructose fed compared to the glucose fed groups (Table 39).

Table 36

Expt. A7 The effect of feeding oxonate-supplemented diets rich in fructose or glucose on body weight and energy intake

	Initial body wt. (g)	Final body wt. (g)	Energy intake (kJ/100 g per day)
High fructose (10)	142 ± 3	185 ± 4	124.7
High fructose plus oxonate (10)	148 ± 4	177 ± 5	108.0
High glucose (10)	148 ± 1	192 ± 3	126.4
High glucose plus oxonate (10)	149 ± 3	184 ± 4	116.2

Results are the Mean ± SEM for the number of observations in parthenesis.

Table 37

Expt. A7 The effect of feeding oxonate-supplemented diets rich in fructose or glucose on plasma uric acid concentration, kaolin-cephalin clotting time and whole blood platelet count

	Plasma uric acid (mg/l)	Kaolin-cephalin clotting time (seconds)	Whole blood platelet count ($\times 10^8$/ml
High fructose	13.6 ± 1.7 (9)	27.1 ± 1.5 (7)	6.4 ± 0.2 (7)
High fructose plus oxonate	20.9 ± 2.5* (10)	26.2 ± 0.9 (6)	6.8 ± 0.3 (6)
High glucose	13.2 ± 1.3 (10)	27.5 ± 1.4 (6)	6.4 ± 0.2 (6)
High glucose plus oxonate	26.8 ± 3.4†† (10)	28.1 ± 1.4 (8)	7.1 ± 0.2† (8)

Results are the Mean ± SEM for the number of observations in parenthesis.

Significance of difference from High fructose - * $p < 0.05$

Significance of difference from High glucose - † $p < 0.05$ †† $p < 0.01$

Table 38

Expt. A7 The effect of feeding oxonate-supplemented diets rich in fructose or glucose on ADP-induced and thrombin-induced aggregation

		VAmax(cm/min per 10^8 platelets)		Amax(cm/10^8 platelets)	
		ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
High fructose	(6)	3.82 ± 0.33	5.18 ± 0.28	3.74 ± 0.12	4.48 ± 0.40
High fructose plus oxonate	(6)	3.71 ± 0.39	5.16 ± 0.33	3.56 ± 0.20	4.59 ± 0.21
High glucose	(6)	3.39 ± 0.11	4.94 ± 0.22	3.56 ± 0.24	4.40 ± 0.28
High glucose plus oxonate	(8)	3.76 ± 0.26	5.14 ± 0.20	3.94 ± 0.20	4.67 ± 0.11

Results are the Mean ± SEM for the number of observations in parenthesis.

Table 39

Expt. A7 The effect of feeding oxonate-supplemented diets rich in fructose or glucose on kidney weight and uric acid concentration

	Kidney wt. (g/100 g body wt.)	Kidney uric acid (mg/100 g body wt.)	Kidney uric acid (mg/g tissue per 100 g body wt.)
High fructose (10)	0.78 ± 0.03	0.31 ± 0.03	0.22 ± 0.02
High fructose plus oxonate (10)	0.79 ± 0.03	0.35 ± 0.03	0.25 ± 0.02
High glucose (10)	0.67 ± 0.02**	0.28 ± 0.02	0.22 ± 0.02
High glucose plus oxonate (10)	0.68 ± 0.02††	0.33 ± 0.02	0.27 ± 0.03

Results are the Mean ± SEM for the number of observations in parenthesis.

Significance of difference from High fructose - ** $p < 0.01$

High fructose plus oxonate - †† $p < 0.01$

Table 37A

Expt. A7 The effect of feeding oxonate-supplemented diets rich in fructose or glucose on platelet-rich plasma platelet count (PRP) and on the ratio to whole blood platelet count (WB)

	Platelet-rich plasma platelet count ($\times 10^8$/ml)	Ratio PRP:WB
High fructose (6)	11.8 ± 0.3	1.8
High fructose plus oxonate (6)	12.5 ± 0.5	1.8
High glucose (6)	11.8 ± 0.7	1.8
High glucose plus oxonate (8)	12.5 ± 0.8	1.8

Results are the Mean ± SEM for the number of observations in parenthesis.

Table 38A

Expt. A7 The effect of feeding oxonate-supplemented diets rich in fructose or glucose on ADP-induced and thrombin-induced aggregation (uncorrected)

	VAmax (cm/min)		Amax (cm)	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
High fructose (6)	9.02±0.86	12.23±0.80	8.81±0.34	10.54±0.89
High fructose plus oxonate (6)	9.38±1.14	13.05±1.23	9.00±0.88	11.52±0.72
High glucose (6)	8.01±0.63	11.65±0.95	8.40±0.83	10.42±1.11
High glucose plus oxonate (8)	9.48±1.05	12.87±0.94	9.87±0.81	11.75±0.92

Results are the Mean ± SEM for the number of observations in parenthesis.

Sections B, C and D

The effect of alterations in dietary protein, fat and carbohydrate on platelet aggregation

Introduction

Arterial thrombosis is the main lethal complication of atherosclerosis in cardiovascular disease, and there is increasing evidence that thrombosis may play a role in atherogenesis (Hornstra 1975).

Coronary heart disease is extremely uncommon in most tropical and subtropical countries, except among those small sections of the community, who are affluent and following a way of life resembling that found in most economically developed countries (Shaper 1974). One of the most striking differences between communities of developing and developed countries showing such diversity of susceptibility to ischaemic heart disease is in the dietary habits and in particular the proportion of total energy obtained from protein, fat and carbohydrate. It is therefore valuable to study the effect of dietary alterations on arterial thrombus formation and more particularly on blood platelet function, which is an essential component of thrombus formation. However there seems to be little uniformity in the methods used to test platelet function under different dietary conditions and this is probably a major reason for the variation in the results reported.

Tests of platelet function fall into two main categories. Firstly those tests which measure platelet adhesiveness,which is the ability of platelets to stick to a foreign surface and tests of adhesiveness are based on one of two original methods. Wright's method (1941) measures the fall in platelet count when citrated or heparinised blood is rotated in a glass container under standard conditions, whereas Hellem's original method (1960) determines the percentage of platelets retained, when citrated blood is passed through a glass-bead column. 'Adhesiveness' is the term used to describe the results obtained with Wright's test and 'retention' used to describe results obtained with glass-bead column methods. Results from both Wright's (1941) and Hellem's (1960) tests of platelet adhesiveness are influenced by haematocrit, flow velocity, anticoagulant, timing and mixing procedures,

age of glass-bead column and the type of plastic used. Salzman (1963) also suggested that both the adhesion of platelets to glass and the sticking of platelets to each other are involved in tests of retention. The glass bead column acts as a filter of platelet aggregates and measures a degree of aggregation as well as adhesion. The initial adhesion processes cannot be validly imitated outside the vessel wall so that the mechanism is difficult to investigate experimentally. Shaper (1974) reported that there were no differences in platelet adhesiveness, as measured by the methods of Wright (1941) and Hellem (1960), between middle-aged European, Asian and African men living in Uganda, even though coronary heart disease is a major cause of death in Asians and Europeans living in East Africa, but is virtually non-existent in the African population. It was suggested that platelet adhesiveness as measured by these methods was of doubtful importance in distinguishing between coronary-prone and non-susceptible groups.

The second main type of test of platelet function that has been used to study the effect of dietary alterations, is one which measures platelet aggregation, which is the sticking of platelets to each other. This test measures the change in optical density in a suspension of platelets either in plasma or in isotonic saline, which occurs when an aggregating agent is added (Born 1963). Aggregation can be observed in vitro more readily than adhesion and therefore much more is known about aggregation than about adhesion and this test is the one used in the present studies.

In view of the implications of dietary involvement in the aetiology of ischaemic heart disease suggested above, a study has been carried out in rats to examine the effects of alterations in the protein, fat and carbohydrate content of the diet on platelet function, which is an essential component of thrombus formation. The test of platelet function used was one which measures platelet aggregation, which, as suggested above, is considered a more useful in vitro test of platelet function than tests, which measure platelet adhesiveness.

In Section B are described experiments in which casein is the protein used and the effect on platelet aggregation of different levels of protein included in the diet and the duration for which they are given is compared to a group of rats maintained continuously on a control diet containing 200 g casein/kg diet.

In Section C are described experiments in which alterations in the proportion of total energy supplied by fat and carbohydrate were made. In Expt. C1 alterations were made in both the amount and type of fat included in the diet using safflower oil as a source of poly-unsaturated fat and coconut oil as a source of highly saturated fats and the effects on platelet aggregation were examined. In Expts C2 and 3 experiments are described in which the amount and type of carbo-hydrate included in the diet was altered and the effects on platelet aggregation examined.

In Section D an experiment is described, which was carried out to examine the possible interaction between the effects of dietary protein, fat and carbohydrate on platelet aggregation.

Section B

Expt. B1 The effect of level of protein in the diet

Four groups of female rats were fed on diets containing 50, 90, 130 or 200 g casein/kg diet for 21 d prior to investigation following an initial period of 10 d on stock diet. (Table 6)

Final body weight was reduced when 90 g casein/kg diet was included ($p < 0.05$) and further reduced when 50 g casein/kg diet was included ($p < 0.001$) compared to the control group (200 g casein/kg diet) (Table 40).

There was a progressive reduction in kaolin-cephalin clotting time with decreasing amounts of dietary casein (130 g casein/kg diet 10%; 90 g casein/kg diet 18%; 50 g casein/kg diet 21%) compared to the controls (200 g casein/kg diet). Platelet count in the blood of rats given the diet containing 50 g casein/kg diet was significantly reduced ($p < 0.001$) compared with control values, but no significant changes occurred with the other experimental diets (Table 40).

ADP was the only aggregating agent used in this experiment. The maximum rate of aggregation (VAmax) was reduced from 6.01 cm/min per 10^8 platelets in the rats fed the control diet (200 g casein/kg diet) to 3.80 cm/min per 10^8 platelets in the animals fed 50 g casein/kg diet ($p < 0.01$). No significant differences for VAmax values were observed between the control and other experimental groups. A similar reduction was observed for the maximum extent of aggregation (Amax) between the group fed 50 g casein/kg diet ($p < 0.01$) and the control group, but no other differences were shown for any of the other groups. (Table 41).

Plasma albumin concentration was reduced in animals given the 90 g casein/kg diet ($p < 0.05$) and in those animals given the 50 g casein/kg diet ($p < 0.05$) compared to those rats on the control diet. There was a 23% reduction in the plasma triglyceride concentration in animals given a diet containing 90 g casein/kg diet compared with the controls and remained low when animals were given 50 g casein/kg diet. Plasma cholesterol concentration was increased 16% in animals given 50 g casein/kg diet ($p < 0.05$) compared to control animals, and increased by 11% in animals given 90 g casein/kg diet although non-significantly (Table 42).

Table 40

Expt. B1 The effect of the level of protein in the diet on body weight, energy intake, kaolin-cephalin clotting time and whole blood platelet count.

Casein in diet (g/kg)	Initial body wt. (g)	Final body wt. (g)	Energy intake kJ/100 g per day)	Kaolin-cephalin clotting time (seconds)	Whole blood platelet count ($\times 10^8$/ml)
200 (Control)	111 ± 2 (10)	180 ± 3 (10)	183.3 (10)	26.6 ± 0.9 (9)	6.5 ± 0.2 (9)
130	113 ± 3 (10)	176 ± 4 (10)	203.1 (10)	23.9 ± 0.8* (9)	6.5 ± 0.2 (9)
90	114 ± 3 (10)	164 ± 5* (10)	215.1 (10)	21.7 ± 0.5***(9)	6.1 ± 0.2 (9)
50	109 ± 3 (10)	124 ± 4***(10)	220.7 (10)	20.9 ± 1.0***(9)	5.1 ± 0.2***(9)

Results are the Mean ± SEM for the number of observations in parenthesis.

Significance of difference from Control *p < 0.05 **p < 0.01 ***p < 0.001

Table 41

Expt. B1 The effect of the level of protein in the diet on ADP-induced aggregation

Casein in diet (g/kg)	ADP-induced aggregation	
	VAmax (cm/min per 10^8 platelets)	Amax (cm/10^8 platelets)
200 (9) (Control)	6.01 ± 0.40	5.01 ± 0.20
130 (9)	5.56 ± 0.54	4.95 ± 0.22
90 (9)	5.37 ± 0.51	4.94 ± 0.33
50 (7)	3.80 ± 0.39**	4.05 ± 0.25**

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from Control **p < 0.01

Table 42

Expt. B1 The effect of the level of protein in the diet on plasma albumin, triglyceride and cholesterol concentrations

Casein in diet (g/kg)	Plasma albumin (g/l)	Plasma triglyceride (mg/l)	Plasma cholesterol (mg/l)
200 (Control)	25.9 ± 0.9	552 ± 31	547 ± 22
130	26.9 ± 2.0	523 ± 38	572 ± 25
90	23.7 ± 0.5*	408 ± 19**	622 ± 37
50	23.1 ± 0.8*	424 ± 26**	634 ± 21*

Results are the Mean ± SEM for nine rats per treatment.
Significance of difference from Control *p < 0.05 **p < 0.01

Table 40A

Expt. B1 The effect of the level of protein in the diet on platelet-rich plasma platelet count (PRP) and on the ratio to whole blood platelet count (WB)

Casein in diet (g/kg)	Platelet-rich plasma platelet count ($\times 10^8$/ml)	Ratio PRP:WB
200 (9) Control	14.4 ± 0.6	2.2
130 (9)	13.9 ± 0.5	2.1
90 (9)	13.2 ± 0.6	2.2
50 (7)	10.4 ± 0.6***	2.0

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from Control - *** $p < 0.001$

Table 41A

Expt. B1 The effect of the level of protein in the diet on ADP-induced aggregation (uncorrected)

Casein in diet (g/kg)	ADP-induced aggregation	
	VAmax (cm/min)	Amax (cm)
200 (9) Control	17.59 ± 1.65	14.54 ± 0.94
130 (9)	15.53 ± 1.73	13.81 ± 0.88
90 (9)	14.34 ± 1.61	13.20 ± 1.15
50 (7)	7.98 ± 1.01***	8.50 ± 0.82***

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from Control - *** $p < 0.001$

Expt. B2 The effect of duration on low protein diet

It was established in Expt. B1 that rats fed on diets containing 50 g casein/kg diet for 21 d had a reduced ADP-induced platelet aggregation compared to animals fed the control diet (200 g casein/kg diet). An experiment was therefore carried out to examine if a similar response occurred with thrombin-induced aggregation and to investigate the time course of the effect of low protein diet on ADP-induced and thrombin-induced aggregation. Four groups of female rats were fed initially for 25 d following weaning on stock diet. Animals were then fed on a diet containing 50 g casein/kg diet for periods of 0, 4, 12 or 21 d. When not on low protein diet animals were given a 200 g casein/kg control diet and feeding was arranged so that all groups finished their period on low protein diets at the same time (Table 7). Data presented in the control (0 d) and the 21 d groups includes values from another trial performed on a different occasion, but carried out under identical conditions and which produced similar values for each of the variables studied.

No significant differences for kaolin-cephalin clotting time (KCCT) were observed between the animals given the control diet (0 d) and those given the low protein diet, even when given for 21 d. This finding casts some suspicion on the results for KCCT observed in Expt. B1 where a progressive reduction in KCCT values was seen with decreasing protein content of the diet compared to the control diet (200 g casein/kg diet). Whole blood platelet count was reduced after 4 d on the low protein diet ($p < 0.01$) and remained reduced in animals given low protein diets for longer periods, when compared to those animals given the control diet all the time (Table 43).

There was a progressive fall in the maximum rate of aggregation (VAmax) with increasing time on the low protein diet for both ADP-induced and thrombin-induced aggregation compared to the controls (4 d 13%; 12 d 23%; 21 d 29%). A similar trend existed for the maximum extent of aggregation (Amax) (Table 44).

Table 43

Expt. B2 The effect of duration on low protein diet on body weight, energy intake kaolin-cephalin clotting time and whole blood platelet count

Time on low protein diet (d)	Initial body wt. (g)	Final body wt. (g)	Energy intake (kJ/100 g per day)	Kaolin-cephalin clotting time (seconds)	Whole blood platelet count ($\times 10^8$/ml)
0 (Control)	166 ± 4 (25)	197 ± 4 (25)	134.9 (25)	31.1 ± 1.5 (19)	6.6 ± 0.2 (19)
4	172 ± 7 (10)	198 ± 7 (10)	139.3 (10)	27.6 ± 2.0 (8)	5.4 ± 0.2** (8)
12	164 ± 6 (10)	186 ± 5 (10)	151.4 (10)	26.6 ± 1.0 (8)	5.9 ± 0.1 (8)
21	160 ± 3 (25)	178 ± 6* (25)	164.8 (25)	29.6 ± 1.2 (10)	5.1 ± 0.4***(10)

Results are the Mean ± SEM for the number of observations in parenthesis.

Significance of difference from Control values *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$

Table 44

Expt. B2 The effect of duration on low protein diet on ADP-induced and thrombin-induced aggregation.

Time on low protein diet (d)	VAmax(cm/min per 10^8 platelets)		Amax(cm/10^8 platelets)	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
0(19)(Control)	4.80±0.16	6.15±0.19	4.69±0.09	5.66±0.18
4(8)	4.18±0.21*	4.87±0.31*	4.03±0.25	4.83±0.17*
12(8)	3.68±0.15**	4.67±0.11**	3.47±0.15***	4.29±0.17***
21(10)	3.42±0.21***	4.59±0.28***	3.92±0.16***	4.57±0.19***

Results are the Mean ± SEM for the number of observations in parenthesis. Significance of difference from Control values *$p < 0.05$ **$p < 0.01$ ***$p < 0.001$.

Table 43A

Expt. B2 The effect of duration on low protein diet on platelet-rich plasma platelet count (PRP) and on the ratio to whole blood platelet count (WB)

Time on low protein diet (d)	Platelet-rich plasma platelet count ($\times 10^8$/ml)	Ratio PRP:WB
0 (19) Control	13.2 ± 0.5	2.0
4 (8)	11.6 ± 0.7	2.1
12 (8)	11.1 ± 0.4*	1.9
21 (10)	8.8 ± 0.7***	1.7

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from Control - * $p < 0.02$ *** $p < 0.001$

Table 44A

Expt. B2 The effect of duration on low protein diet on ADP-induced and Thrombin-induced aggregation (uncorrected)

Time on low protein diet (d)	VAmax (cm/min)		Amax(cm)	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
0 (19) Control	12.82±0.77	16.34±0.91	12.45±0.59	15.07±0.83
4 (8)	9.71±0.82*	11.41±1.17**	9.42±0.89**	11.34±1.02*
12 (8)	8.19±0.53**	10.36±0.53***	7.71±0.48***	9.51±0.50***
21 (10)	6.20±0.83***	8.22±1.02***	7.00±0.77***	8.06±0.76***

Results are the Mean < SEM for the number of observations in parenthesis.
Significance of difference from Control - * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Section C

Expt. C1 The effect of feeding diets rich in unsaturated or saturated fat

Four groups of female rats were fed for 21 d prior to investigation on diets containing high or low levels of safflower (highly unsaturated) or coconut (highly saturated) oil (Table 8) following an initial period of 10 d on stock diet. Data from animals given diets containing high levels of safflower or coconut oil also contains results from a trial performed on a different occasion, but carried out under identical conditions and which produced similar values for the variables studied. Statistical treatment of data was performed by two factorial analysis of variance and the results of this are presented below each table of data to which they are related.

Analysis of variance revealed no significant effect due to either the level of fat or the type of fat in the diet in live weight gain, kaolin-cephalin clotting time or whole blood platelet count. (Table 45).

No differences were observed between groups for any of the aggregation studies carried out (Table 46).

Plasma triglyceride concentration was increased in the group fed on the diet high in coconut oil, when compared to either the low coconut group ($p < 0.01$) or the high safflower group ($p < 0.001$). There was a significant increase in plasma cholesterol concentration in animals given diets containing coconut oil compared to animals given safflower oil, ($p < 0.001$), but the level of fat given had no effect (Table 47).

Expt. C2 The effect of the level of sucrose in the diet

Two groups of female rats were fed on diets containing 50 or 400 g sucrose/kg diet replacing starch (Table 9) for 21 d prior to investigation following an initial period of 10 d on stock diet.

The final body weight was reduced in animals given 400 g sucrose/kg diet ($p < 0.001$) due to a 10% reduction in energy intake in the group compared with animals given the diet containing 50 g sucrose/kg diet. There was a 29% increase in kaolin-cephalin clotting time in the group given the diet rich in sucrose compared to animals given the diet poor in sucrose. No differences in the whole blood platelet count were

Table 45

Expt. C1 The effect of feeding diets rich in unsaturated or saturated fat on body weight, energy intake, kaolin-cephalin clotting time and whole blood platelet count

	Initial body wt. (g)	Final body wt. (g)	Energy intake (kJ/100 g per day)	Kaolin-cephalin clotting time (seconds)	Whole blood platelet count ($\times 10^8$/ml)
Low safflower oil	115 ± 5 (10)	172 ± 3 (10)	170.7 (10)	22.7 ± 0.9 (8)	6.8 ± 0.3 (8)
High safflower oil	114 ± 1 (30)	172 ± 3 (30)	191.4 (30)	22.1 ± 0.4 (26)	6.9 ± 0.1 (26)
Low coconut oil	118 ± 4 (10)	174 ± 5 (10)	179.8 (10)	21.6 ± 0.7 (10)	6.8 ± 0.2 (10)
High coconut oil	115 ± 2 (30)	170 ± 3 (30)	187.6 (30)	21.8 ± 0.5 (28)	7.2 ± 0.1 (28)

Results are the Mean ± SEM for the number of observations in parenthesis.

Analysis of Variance

	Live weight gain	Kaolin-cephalin clotting time	Whole blood platelet count
Type of fat	NS	NS	NS
Level of fat	NS	NS	NS
Interaction	NS	NS	NS

Table 46

Expt. C1 The effect of feeding diets rich in unsaturated or saturated fat on ADP-induced aggregation

	ADP-induced aggregation	
	VAmax (cm/min per 10^8 platelets)	Amax (cm/10^8 platelets)
Low safflower oil (8)	5.26 ± 0.42	5.28 ± 0.34
High safflower oil (26)	5.40 ± 0.19	5.06 ± 0.11
Low coconut oil (9)	5.53 ± 0.44	5.21 ± 0.19
High coconut oil (26)	5.03 ± 0.22	4.78 ± 0.16

Results are the Mean ± SEM for the number of observations in parenthesis.

Analysis of Variance

	ADP-induced aggregation	
	VAmax	Amax
Type of fat	NS	NS
Level of fat	NS	NS
Interaction	NS	NS

Table 45A

Expt. C1 The effect of feeding diets rich in unsaturated or saturated fat on platelet-rich plasma platelet count (PRP) and on the ratio to whole blood platelet count (WB)

		Platelet-rich plasma platelet count ($\times 10^8$/ml)	Ratio PRP:WB
Low safflower oil	(8)	14.6 ± 0.7	2.1
High safflower oil	(26)	15.1 ± 0.3	2.2
Low coconut oil	(9)	14.9 ± 0.6	2.2
High coconut oil	(26)	15.6 ± 0.4	2.2

Results are the Mean ± SEM for the number of observations in parenthesis.

Table 46A

Expt. C1 The effect of feeding diets rich in unsaturated or saturated fat on ADP-induced aggregation (uncorrected)

		ADP-induced aggregation	
		VAmax (cm/min)	Amax (cm)
Low safflower oil	(8)	15.53 ± 1.54	15.47 ± 1.25
High safflower oil	(26)	16.30 ± 0.64	15.29 ± 0.43
Low coconut oil	(9)	16.50 ± 1.63	15.24 ± 0.76
High coconut oil	(26)	16.77 ± 0.81	15.47 ± 0.52

Results are the Mean ± SEM for the number of observations in parenthesis.

Table 47

Expt. C1 The effect of feeding diets rich in unsaturated or saturated fat on plasma triglyceride and cholesterol concentrations

		Plasma triglyceride (mg/l)	Plasma cholesterol (mg/l)
Low safflower oil	(9)	551 ± 48	522 ± 17
High safflower oil	(27)	543 ± 38	498 ± 12
Low coconut oil	(10)	594 ± 50	580 ± 21
High coconut oil	(28)	1005 ± 64	620 ± 15

Results are the Mean ± SEM for the number of observations in parenthesis.

Analysis of variance

	Plasma triglyceride	Plasma cholesterol
Type of fat	***	***
Level of fat	**	NS
Interaction	**	NS

Significance of difference **p < 0.01 ***p < 0.001

observed between the two groups (Table 48).

There was a small, non-significant decrease in the maximum rate of aggregation (VAmax) (11% decrease) and a larger significant decrease in the maximum extent of aggregation (Amax) (15% decrease; $p < 0.05$) for ADP-induced aggregation in the group given 400 g sucrose/kg diet compared to those animals given 50 g sucrose/kg diet. This decreased ADP-induced aggregation tendency when animals were given a high sucrose diet compared to those animals given the low sucrose diet, was not observed for thrombin-induced aggregation, in fact there were small, non-significant increases in the VAmax (6% increase) and Amax (8% increase) values for thrombin-induced aggregation in the group given the diet containing 400 g sucrose/kg diet compared to those animals given 50 g sucrose/kg diet (Table 49).

Plasma triglyceride concentrations were significantly increased in animals given the diet rich in sucrose compared to those animals given the diet poor in sucrose ($p < 0.01$). No significant changes were observed in plasma cholesterol concentrations between the groups. (Table 50).

Expt. C3 The effect of feeding high or low levels of fructose or glucose

Four groups of female rats were fed on diets containing fructose or glucose at levels 50 or 400 g/kg diet replacing starch for 21 d prior to investigation following an initial period of 10 d on stock diet (Table 10). Statistical treatment of data was performed by two factorial analysis of variance and the results of this are presented below each table of data to which they are related.

Analysis of variance revealed no significant changes, due to either the type of carbohydrate or the level of carbohydrate, in the live weight gain, kaolin-cephalin clotting time, or whole blood platelet count (Table 51).

There were small, non-significant decreases in the maximum rate of aggregation (VAmax) and the maximum extent of aggregation (Amax) for both ADP-induced and thrombin-induced aggregation when animals were given diets containing high levels of fructose or glucose compared to those animals given low fructose or glucose diets (Table 52). Results from Expt. C2 also suggested a lower ADP-induced aggregation

Table 48

Expt. C2 The effect of the level of sucrose in the diet on body weight, energy intake, kaolin-cephalin clotting time and whole blood platelet count.

Sucrose in diet (g/kg)	Initial body wt. (g)	Final body wt. (g)	Energy intake (kJ/100 g per day)	Kaolin-cephalin clotting time (seconds)	Whole blood platelet count ($\times 10^8$/ml)
50	113 ± 3 (30)	171 ± 2 (30)	179.5 (30)	24.2 ± 0.9 (25)	6.8 ± 0.1 (26)
400	107 ± 2 (30)	158 ± 2***(30)	161.1 (30)	31.3 ± 1.0***(25)	7.2 ± 0.2 (25)

Results are the Mean ± SEM for the number of observations in parenthesis.

Significance of difference from 50 g/kg values ***$p < 0.001$

Table 49

Expt. C2 The effect of the level of sucrose in the diet on ADP-induced and thrombin-induced aggregation

Sucrose in diet (g/kg)	VAmax(cm/min per 10^8 platelets)		Amax(cm/10^8 platelets)	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
50	5.02 ± 0.26(25)	5.43 ± 0.68 (8)	5.06±0.17 (24)	5.07 ± 0.34 (6)
400	4.46 ± 0.27(25)	5.74 ± 0.35 (22)	4.40±0.18*(23)	5.49 ± 0.36 (14)

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from 50 g/kg values *p < 0.02

Table 50

Expt. C2 The effect of the level of sucrose in the diet on plasma triglyceride and cholesterol concentrations

Sucrose in diet (g/kg)	Plasma triglyceride (mg/l)	Plasma cholesterol (mg/l)
50 (27)	573 ± 27	563 ± 13
400 (25)	788 ± 60**	586 ± 13

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from 50 g/kg values **p < 0.01

Table 48A

Expt. C2 The effect of the level of sucrose in the diet on platelet-rich plasma platelet count (PRP) and on the ratio to whole blood platelet count (WB)

Sucrose in diet (g/kg)	Platelet-rich plasma platelet count ($\times 10^8$/ml)	Ratio PRP:WB
50 (25)	14.5 ± 0.4	2.1
400 (25)	16.2 ± 0.7*	2.2

Results are the Mean ± SEM for the number of observations in parenthesis.
Significance of difference from 50 g/kg values - * $p < 0.05$

Table 49A

Expt. C2 The effect of the level of sucrose in the diet on ADP-induced and thrombin-induced aggregation (uncorrected)

Sucrose in diet (g/kg)	VAmax (cm/min)		Amax (cm)	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
50	14.85±1.01(25)	15.93±2.78 (8)	14.69±0.68(24)	13.60±1.50(6)
400	14.49±1.01(25)	18.23±1.33(22)	13.79±0.76(23)	15.26±1.13(14)

Results are the Mean ± SEM for the number of observations in parenthesis.

tendency in the high sugar (sucrose) group than in the low sugar group, although values for thrombin-induced aggregation suggested an effect in the opposite direction.

There was a significant effect on plasma triglyceride concentration due to the type of carbohydrate in the diet ($p < 0.05$); fructose produced higher plasma triglyceride concentrations than glucose. The significant interaction reflects the fact that raising the dietary level of fructose increased the plasma triglyceride concentration whereas the opposite effect was observed for glucose. In Expt. C2 a higher plasma triglyceride concentration was observed in the group fed on the diet high in sucrose compared to those animals given the low sucrose diet. This suggests that it is the fructose moiety of sucrose, which is associated with the increased plasma triglyceride concentration, which occurred in animals fed the sucrose-rich diet. No significant changes were observed in plasma cholesterol concentrations due to either the type of carbohydrate or the level of carbohydrate (Table 53).

Table 51

Expt. C3 The effect of feeding high or low levels of fructose or glucose in the diet on body weight, energy intake, kaolin-cephalin clotting time and whole blood platelet count

	Initial body wt. (g)	Final body wt. (g)	Energy intake (kJ/100 g per day)	Kaolin-cephalin clotting time (seconds)	Whole blood platelet count ($\times 10^8$/ml)
High fructose	101 ± 2 (10)	154 ± 2 (10)	163.5 (10)	30.1 ± 1.5 (7)	6.3 ± 0.3 (7)
Low fructose	100 ± 3 (10)	159 ± 3 (10)	160.2 (10)	31.5 ± 1.1 (6)	6.9 ± 0.3 (6)
High glucose	99 ± 3 (10)	156 ± 3 (10)	164.5 (10)	30.5 ± 1.1 (9)	6.4 ± 0.2 (9)
Low glucose	97 ± 2 (10)	151 ± 2 (10)	155.5 (10)	30.4 ± 1.3 (6)	6.1 ± 0.4 (6)

Results are the Mean ± SEM for the number of observations in parenthesis.

Analysis of Variance

	Live weight gain	Kaolin-cephalin clotting time	Whole blood platelet count
Type of carbohydrate	NS	NS	NS
Level of carbohydrate	NS	NS	NS
Interaction	NS	NS	NS

Table 52

Expt. C3 The effect of feeding high or low levels of fructose or glucose in the diet on ADP-induced and thrombin-induced aggregation

		VAmax(cm/min per 10^8 platelets)		Amax(cm/10^8 platelets)	
		ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
High fructose	(7)	4.19 ± 0.29	5.06 ± 0.31	4.58 ± 0.26	5.80 ± 0.20
Low fructose	(6)	4.65 ± 0.44	6.26 ± 0.58	4.60 ± 0.12	5.84 ± 0.22
High glucose	(9)	4.37 ± 0.44	5.23 ± 0.35	4.57 ± 0.33	5.39 ± 0.22
Low glucose	(6)	4.78 ± 0.18	5.53 ± 0.23	4.78 ± 0.22	5.72 ± 0.18

Results are the Mean ± SEM for the number of observations in parenthesis.

Analysis of variance

	VAmax		Amax	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
Type of carbohydrate	NS	NS	NS	NS
Level of carbohydrate	NS	NS	NS	NS
Interaction	NS	NS	NS	NS

Table 51A

Expt. C3 The effect of feeding high or low levels of fructose or glucose in the diet on platelet-rich plasma platelet count (PRP) and on the ratio to whole blood platelet count (WB)

		Platelet-rich plasma platelet count ($\times 10^8$/ml)	Ratio PRP:WB
High fructose	(7)	12.5 ± 0.5	2.0
Low fructose	(6)	13.7 ± 1.3	2.0
High glucose	(9)	12.6 ± 0.6	2.0
Low glucose	(6)	11.9 ± 1.1	2.0

Results are the Mean ± SEM for the number of observations in parenthesis.

Table 52A

Expt. C3 The effect of feeding high or low levels of fructose or glucose in the diet on ADP-induced and thrombin-induced aggregation (uncorrected)

	VAmax (cm/min)		Amax (cm)	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
High fructose (7)	10.53±1.00	12.74±1.19	11.46±0.88	14.41±0.61
Low fructose (6)	13.28±2.60	17.80±3.27	12.71±1.40	14.87±1.34
High glucose (9)	11.16±1.40	13.39±1.44	11.62±1.12	13.21±0.75
Low glucose (6)	11.48±1.42	13.36±1.83	11.44±1.32	13.65±1.42

Results are the Mean ± SEM for the number of observations in parenthesis.

Table 53

Expt. C3 The effect of feeding high or low levels of fructose or glucose in the diet on plasma triglyceride and cholesterol concentrations

	Plasma triglyceride (mg/l)	Plasma cholesterol (mg/l)
High fructose	647 ± 45	539 ± 19
Low fructose	556 ± 32	544 ± 17
High glucose	460 ± 21	544 ± 21
Low glucose	565 ± 25	502 ± 17

Results are the Mean ± SEM for ten rats per treatment

Analysis of variance

	Plasma triglyceride	Plasma cholesterol
Type of carbohydrate	*	NS
Level of carbohydrate	NS	NS
Interaction	**	NS

Significance of difference $^{*}p < 0.05$ $^{**}p < 0.01$

Section D

The interaction between the levels of casein, safflower oil and sucrose included in the diet

In Sections B and C experiments are described in which the effects of alterations in the level of dietary protein or the level and type of dietary fat or carbohydrate on platelet aggregation were examined. In the experiment described below the effects of simultaneous alterations in the levels of dietary protein, fat and carbohydrate on platelet aggregation were examined in order to study the possible interactions between these dietary components in their effect on platelet aggregation in rats. Casein and sucrose were the protein and sugar sources respectively and were included in the diet at the expense of maize starch. Expt. C1 established no significantly different effects on platelet aggregation between safflower oil and coconut oil when included in the diet as the fat source. For this reason safflower oil was the only fat used in this experiment.

Eight groups of female rats were given diets containing 50 or 200 g casein, 50 or 200 g safflower oil and 50 or 400 g sucrose/kg for 21 d prior to investigation following an initial period of 10 d on stock diet (Table 11). Data was analysed statistically by two factorial analysis of variance for unequal cell numbers and by students 't' test.

Analysis of variance revealed that final body weight was significantly reduced in the low protein groups ($p < 0.001$) compared to the high protein groups regardless of the levels of safflower oil or sucrose included in the diet (Table 54).

There were no significant differences in kaolin-cephalin clotting time between any of the groups. Whole blood platelet count was significantly reduced in the low casein groups, when compared to the high casein groups, regardless of the sucrose or safflower oil content of the diets ($p < 0.001$). Among the low-protein, groups there was an effect due to the level of fat in the diet on whole blood platelet count; in animals given diets containing a low level of safflower oil whole blood platelet count was lower compared to those animals given a high level of safflower oil ($p < 0.05$) (Table 55).

The maximum rate of aggregation (VAmax) for both ADP-induced and thrombin-induced aggregation was reduced in animals given low casein

diets, compared to those animals given the diets high in casein, regardless of the sucrose or safflower oil content of the diets ($p < 0.001$). Among the low-casein, low-sucrose groups there was a significant increase in VAmax for ADP-induced aggregation in animals given diets high in safflower oil compared to those animals given diets containing low levels of safflower oil ($p < 0.05$). There was a significant reduction in VAmax for ADP-induced aggregation in animals given the diets containing a high level of sucrose compared to those animals given the low sucrose diet, when the diets also contained high safflower oil and low casein ($p < 0.05$). The maximum extent of aggregation (Amax) for both ADP-induced and thrombin-induced aggregation was reduced in animals given low levels of casein in the diet compared to the high-casein groups, regardless of the sucrose ($p < 0.001$) or the safflower oil ($p < 0.01$) content of the diet. Among the low-sucrose, low-casein groups, Amax for ADP-induced and thombin-induced aggregation was increased in those animals given diets containing high levels of safflower oil compared to animals given low safflower oil diets. ($p < 0.05$). At high protein no significant effects on ADP-induced or thrombin-induced aggregation were observed, although there was a trend towards high-sucrose diets producing lower aggregation values than the low-sucrose groups. (Table 56).

There was a significant reduction in plasma triglyceride concentration in animals given diets rich in sucrose compared to those animals given diets poor in sucrose, when diets were also rich in safflower oil regardless of the protein content of the diet ($p < 0.05$). Plasma cholesterol concentration was reduced in animals given high levels of safflower oil in the diet compared to the low safflower oil groups, when diets also contained a low level of casein, regardless of the sucrose content of the diet ($p < 0.001$) (Table 57).

Table 54

Expt. D1 The effect of the interaction between the levels of casein, safflower oil and sucrose in the diet on body weight and energy intake

	Initial body wt. (g)	Final body wt. (g)	Energy intake (kJ/100 g per day)
High safflower oil High casein High sucrose	109 ± 3	160 ± 4	160.6
High safflower oil High casein Low sucrose	109 ± 3	163 ± 4	171.5
High safflower oil Low casein High sucrose	107 ± 3	97 ± 3	167.9
High safflower oil Low casein Low sucrose	107 ± 3	92 ± 4	160.9
Low safflower oil High casein High sucrose	110 ± 3	164 ± 4	151.7
Low safflower oil High casein Low sucrose	108 ± 2	158 ± 3	157.0
Low safflower oil Low casein High sucrose	106 ± 3	98 ± 4	162.9
Low safflower oil Low casein Low sucrose	108 ± 3	111 ± 3	194.8

Results are the Mean ± SEM for fifteen rats per treatment

Table 55

Expt. D1 The effect of the interaction between the levels of casein, safflower oil and sucrose in the diet on kaolin-cephalin clotting time and whole blood platelet count

		Kaolin-cephalin clotting time (seconds)	Whole blood platelet count ($\times 10^8$/ml)
High safflower oil High casein High sucrose	(8)	30.4 ± 2.0	7.3 ± 0.2
High safflower oil High casein Low sucrose	(10)	32.1 ± 1.4	6.8 ± 0.2
High safflower oil Low casein High sucrose	(9)	28.1 ± 2.3	5.1 ± 0.1
High safflower oil Low casein Low sucrose	(6)	30.3 ± 2.9	5.6 ± 0.3
Low safflower oil High casein High sucrose	(12)	32.0 ± 1.1	6.8 ± 0.2
Low safflower oil High casein Low sucrose	(11)	32.8 ± 2.3	7.2 ± 0.2
Low safflower oil Low casein High sucrose	(7)	29.1 ± 1.8	5.3 ± 0.3
Low safflower oil Low casein Low sucrose	(6)	30.2 ± 1.9	4.4 ± 0.2

Results are the Mean ± SEM for the number of observations in parenthesis.

Table 55A

Expt. D1 The effect of the interaction between the levels of casein, safflower oil and sucrose in the diet on platelet-rich plasma platelet count (PRP) and on the ratio to whole blood platelet count (WB)

		Platelet-rich plasma platelet count ($\times 10^8$/ml)	Ratio PRP:WB
High safflower oil High casein High sucrose	(8)	14.8 ± 0.6	2.0
High safflower oil High casein Low sucrose	(10)	13.1 ± 0.5	1.9
High safflower oil Low casein High sucrose	(9)	9.2 ± 0.4	1.8
High safflower oil Low casein Low sucrose	(6)	10.9 ± 0.9	1.9
Low safflower oil High casein High sucrose	(12)	13.1 ± 0.6	1.9
Low safflower oil High casein Low sucrose	(11)	13.7 ± 0.6	1.9
Low safflower oil Low casein High sucrose	(7)	9.9 ± 0.6	1.9
Low safflower oil Low casein Low sucrose	(6)	7.4 ± 0.4	1.7

Results are the Mean ± SEM for the number of observations in parenthesis.

Expt. D1	Analysis of variance Platelet-rich plasma platelet count
At high safflower oil	
Level of sucrose	NS
Level of casein	***
Interaction	**
At low safflower oil	
Level of sucrose	NS
Level of casein	***
Interaction	*
At high casein	
Level of sucrose	NS
Level of safflower oil	NS
Interaction	*
At low casein	
Level of sucrose	NS
Level of safflower oil	*
Interaction	**
At high sucrose	
Level of safflower oil	NS
Level of casein	***
Interaction	*
At low sucrose	
Level of safflower oil	*
Level of casein	***
Interaction	**

Analysis of variance

	Kaolin-cephalin clotting time	Whole blood platelet count	Final body weight
At high safflower oil			
Level of sucrose	NS	NS	NS
Level of casein	NS	***	***
Interaction	NS	*	NS
At low safflower oil			
Level of sucrose	NS	NS	NS
Level of casein	NS	***	***
Interaction	NS	*	NS
At high casein			
Level of sucrose	NS	NS	NS
Level of safflower oil	NS	NS	NS
Interaction	NS	NS	NS
At low casein			
Level of sucrose	NS	NS	NS
Level of safflower oil	NS	*	*
Interaction	NS	**	*
At high sucrose			
Level of safflower oil	NS	NS	NS
Level of casein	NS	***	***
Interaction	NS	NS	NS
At low sucrose			
Level of safflower oil	NS	NS	NS
Level of casein	NS	***	***
Interaction	NS	**	**

Students 't' test

	Casein		Sucrose		Safflower oil	
	High	Low	High	Low	High	Low
Final body wt.	161±2	99±2***	130±5	131±4	128±5	133±4
Kaolin-cephalin clotting time	31.9±0.9	29.3±1.1	30.1±0.9	31.6±1.1	30.2±1.1	31.4±0.9
Whole blood platelet count	7.0±0.1	5.1±0.1***	6.2±0.2	6.3±0.2	6.3±0.2	6.2±0.2

Significance of difference compared with ***$p < 0.001$ corresponding 'high' diet.

Table 56

Expt. D1 The effect of the interaction between the levels of casein, safflower oil and sucrose in the diet on ADP-induced and thrombin-induced aggregation

		VAmax(cm/min per 10^8 platelets)		Amax(cm/10^8 platelets)	
		ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
High safflower oil High casein High sucrose	(8)	4.90±0.30	5.78±0.51	4.58±0.16	5.43±0.20
High safflower oil High casein Low sucrose	(10)	4.98±0.22	6.35±0.42	4.92±0.17	6.00±0.18
High safflower oil Low casein High sucrose	(9)	2.81±0.24	4.24±0.29	3.48±0.25	4.47±0.17
High safflower oil Low casein Low sucrose	(6)	4.07±0.29	4.85±0.34	4.45±0.32	5.00±0.31
Low safflower oil High casein High sucrose	(12)	4.49±0.13	5.72±0.30	4.69±0.13	5.95±0.17
Low safflower oil High casein Low sucrose	(11)	4.84±0.18	6.33±0.22	4.81±0.11	6.06±0.19
Low safflower oil Low casein High sucrose	(7)	3.33±0.24	4.47±0.31	3.68±0.23	4.94±0.29
Low safflower oil Low casein Low sucrose	(6)	3.19±0.28	4.21±0.34	3.79±0.20	4.54±0.27

Results are the Mean ± SEM for the number of observations in parenthesis.

Table 56A

Expt. D1 The effect of the interaction between the levels of casein, safflower oil and sucrose in the diet on ADP-induced and thrombin-induced aggregation (uncorrected)

		VAmax (cm/min)		Amax (cm)	
		ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
High safflower oil High casein High sucrose	(8)	14.65±1.30	17.35±2.02	13.61±0.91	15.63±0.93
High safflower oil High casein Low sucrose	(10)	13.11±0.93	16.67±1.42	12.87±0.63	15.34±0.66
High safflower oil Low casein High sucrose	(9)	5.21±0.55	7.92±0.76	6.47±0.67	8.18±0.46
High safflower oil Low casein Low sucrose	(6)	8.98±1.10	10.62±1.18	9.90±1.35	10.31±1.13
Low safflower oil High casein High sucrose	(12)	11.70±0.57	15.04±1.16	12.17±0.52	14.60±0.69
Low safflower oil High casein Low sucrose	(11)	13.38±0.90	17.40±0.98	13.23±0.65	16.62±0.82
Low safflower oil Low casein High sucrose	(7)	6.70±0.83	9.04±1.10	7.40±0.86	9.97±1.16
Low safflower oil Low casein Low sucrose	(6)	4.75±0.55	6.21±0.56	5.60±0.42	6.69±0.49

Results are the Mean ± SEM for the number of observations in parenthesis.

Expt. D1 — Analysis of variance

	VAmax		Amax	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
At high safflower oil				
Level of sucrose	NS	NS	NS	NS
Level of casein	***	***	***	***
Interaction	*	NS	*	NS
At low safflower oil				
Level of sucrose	NS	NS	NS	NS
Level of casein	***	***	***	***
Interaction	*	*	*	**
At high casein				
Level of sucrose	NS	NS	NS	NS
Level of safflower oil	NS	NS	NS	NS
Interaction	NS	NS	NS	NS
At low casein				
Level of sucrose	NS	NS	NS	NS
Level of safflower oil	NS	NS	NS	NS
Interaction	***	**	**	**
At high sucrose				
Level of safflower oil	NS	NS	NS	NS
Level of casein	***	***	***	***
Interaction	*	NS	NS	NS
At low sucrose				
Level of safflower oil	NS	NS	*	NS
Level of casein	***	***	***	***
Interaction	*	*	**	**

Expt. D1

Students 't' test

	Casein		Sucrose		Safflower oil	
	High	Low	High	Low	High	Low
Platelet-rich plasma platelet count	13.6 ±0.3	9.3 ±0.4***	11.9 ±0.5	11.9 ±0.5	12.0 ±0.5	11.7 ±0.5
VAmax ADP-induced	13.07±0.46	6.29±0.47***	9.76±0.73	10.93±0.74	10.58±0.81	10.08±0.67
VAmax Thrombin-induced	16.52±0.67	8.47±0.54***	12.60±0.91	13.91±0.96	13.35±0.99	13.12±0.89
Amax ADP-induced	12.93±0.33	7.25±0.50***	10.14±0.61	11.13±0.63	10.76±0.65	10.47±0.59
Amax Thrombin-induced	15.58±0.39	8.75±0.50***	12.34±0.68	13.31±0.81	12.72±0.72	12.91±0.76

Significance of difference compared with corresponding 'high' diet - *** $p < 0.001$

Analysis of variance

	VAmax		Amax	
	ADP-induced	Thrombin-induced	ADP-induced	Thrombin-induced
At high safflower oil				
Level of sucrose	*	NS	**	*
Level of casein	***	**	**	***
Interaction	*	NS	NS	NS
AT low safflower oil				
Level of sucrose	NS	NS	**	NS
Level of casein	***	***	**	***
Interaction	NS	NS	NS	NS
At high casein				
Level of sucrose	NS	NS	NS	*
Level of safflower oil	NS	NS	NS	NS
Interaction	NS	NS	NS	NS
At low casein				
Level of sucrose	*	NS	*	NS
Level of safflower oil	NS	NS	NS	NS
Interaction	*	NS	NS	NS
At high sucrose				
Level of safflower oil	NS	NS	NS	NS
Level of casein	***	***	***	***
Interaction	*	NS	NS	NS
AT low sucrose				
Level of safflower oil	*	NS	NS	NS
Level of casein	***	***	***	***
Interaction	NS	NS	NS	NS

Students 't' test

		Casein		Sucrose		Safflower oil	
		High	Low	High	Low	High	Low
VAmax	ADP-induced	4.78±0.10	3.29±0.15***	3.94±0.18	4.44±0.16*	4.20±0.20	4.16±0.15
	Thrombin-induced	6.05±0.18	4.42±0.16***	5.12±0.21	5.68±0.23	5.36±0.25	5.41±0.20
Amax	ADP-induced	4.76±0.07	3.81±0.14***	4.17±0.13	4.60±0.12**	4.36±0.15	4.38±0.11
	Thrombin-induced	5.82±0.10	4.72±0.13***	5.17±0.13	5.58±0.16*	5.30±0.15	5.44±0.15

Significance of difference *p < 0.05 **p < 0.02 ***p < 0.001
compared with corresponding 'high' diet.

Table 57

Expt. D1 The effect of the interaction between the levels of casein, safflower oil and sucrose in the diet on plasma triglyceride and cholesterol concentrations.

		Plasma triglyceride (mg/l)	Plasma cholesterol (mg/l)
High safflower oil High casein High sucrose	(15)	528 ± 19	459 ± 16
High safflower oil High casein Low sucrose	(14)	485 ± 24	463 ± 28
High safflower oil Low casein High sucrose	(10)	618 ± 61	473 ± 12
High safflower oil Low casein Low sucrose	(11)	518 ± 23	498 ± 18
Low safflower oil High casein High sucrose	(15)	558 ± 32	457 ± 25
Low safflower oil High casein Low sucrose	(13)	504 ± 27	478 ± 30
Low safflower oil Low casein High source	(8)	519 ± 29	410 ± 11
Low safflower oil Low casein Low sucrose	(8)	476 ± 22	416 ± 34

Results are the Mean ± SEM for the number of observations in parenthesis

Analysis of variance

	Plasma triglyceride	Plasma cholesterol
At high safflower oil		
Level of sucrose	*	NS
Level of casein	NS	NS
Interaction	NS	NS
At low safflower oil		
Level of sucrose	NS	NS
Level of casein	NS	NS
Interaction	NS	NS
At high casein		
Level of sucrose	NS	NS
Level of safflower oil	NS	NS
Interaction	NS	NS
At low casein		
Level of sucrose	NS	NS
Level of safflower oil	NS	***
Interaction	NS	NS
AT high sucrose		
Level of safflower oil	NS	NS
Level of casein	NS	NS
Interaction	NS	NS
At low sucrose		
Level of safflower oil	NS	NS
Level of casein	NS	NS
Interaction	NS	NS

Students 't' test

	Casein		Sucrose		Safflower oil	
	High	Low	High	Low	High	Low
Plasma triglyceride	520±13	536±21	555±18	497±12**	532±17	520±15
Plasma cholesterol	464±12	455±12	453±10	467±14	472±10	447±14

Significance of difference compared with corresponding 'high' diet. **p < 0.01

CHAPTER 4

DISCUSSION

Section A

Single cell protein and Hyperuricaemia

A Massachusetts Institute of Technology (MIT) conference on single cell protein (SCP) held in 1967 was one of the first to call attention to the problem of the high nucleic acid content of all rapidly growing cells, which if ingested by man, must be excreted mainly as uric acid. Too high an intake of nucleic acid could therefore increase the risk of gouty arthritis in persons with a tendency towards this disease and even precipitate gout in persons not genetically prone to it. It was necessary for this reason to determine the levels of SCP nucleic acid that could be added to the usual diet of populations without an appreciable influence on the risk of hyperuricaemia and gout arising from the deposition of sodium urate crystals in the joints or the formation of uric acid stones in the urinary tract.

Waslien et al (1968) found that, when male human subjects were fed 0, 2, 4 and 8 g. of yeast RNA per day for 5 consecutive days added to otherwise purine-free basal diets, the mean plasma uric acid concentrations were 49, 60, 77 and 94 mg/l respectively. Edozien et al (1970) fed four male MIT students for 9 consecutive days on 0, 45, 90 and 135 g. yeast per day, contributing 0, 2.9, 5.8 and 8.7 g. yeast nucleic acid, in addition to a low-purine basal diet. Plasma uric acid concentrations were 45, 72, 88 and 94 mg/l respectively. On the basis of these results and the fact that 70 mg/l is considered the plasma uric acid concentration, which separates most patients with primary gout from most normal subjects (Grayzel, Liddle and Seegmiller 1961; Gutman and Yü 1957), Edozien et al (1970) proposed a maximum intake of 2 g. SCP nucleic acid per day. The figure of 70 mg/l, which is considered the plasma uric acid concentration defining hyperuricaemia, is based upon statistical analysis of the distribution of plasma uric acid concentration in Western Europe and North America. However, a more logical definition of hyperuricaemia might be one based upon the limited solubility of sodium urate in extracellular fluid. Extracellular fluid is saturated when the urate concentration reaches about

64 mg/l (Peters, and Van Slyke 1946). Above this concentration extracellular fluids are supersaturated with sodium urate, and the potentiality for precipitation of urate crystals exists. Using this definition of hyperuricaemia, the safe limit of SCP nucleic acid intake might well be less than 2 g. per day.

Pinto and Aranjo Neto (1972) point out that the studies of Waslien et al (1968) and Edozien et al (1970) measured nucleic acid added to an otherwise purine-free diet, whereas the purine content of the usual diets consumed by different populations varies greatly. The data of Udo (1969), based on the addition of various amounts of yeast to the usual diets of students and staff at the MIT, support the proposal for a limit of 2 g RNA/man per day, and this figure was accepted in a 1972 PAG guideline as a provisional tolerance limit.

The plasma and urinary uric acid concentrations of 136 volunteers, fed for 3-6 months 1.5 to 2.0 g RNA daily as SCP in addition to their usual diets, were within the acceptable limits of 80 mg/l and 1000 mg/24 h respectively recommended by a 1975 PAG Working Group. However it was suggested that people with a tendency to gout should include SCP in the list of high purine foods to be avoided. (Scrimshaw 1975).

Waslien et al (1968) fed male subjects purine-free diets containing 0-75 g. protein per day as egg albumin. In subjects given protein-free diets, plasma uric acid concentration was 60 mg/l and urinary uric acid excretion was 354 mg/24 h compared to 47 mg/l and 392 mg/24 h respectively in subjects given 75 g protein per day. A reduced dietary protein intake may therefore result in an increase in the plasma uric acid concentration.

Plasma albumin, the concentration of which has been shown to vary with dietary protein levels, may bind to circulating uric acid (Klinenberg and Kippen 1970). A reduced protein intake could therefore lead to a reduction in plasma albumin concentration, which may result in a reduced binding of albumin to uric acid and an increase in plasma free uric acid concentration. In this way a reduced dietary protein intake may influence the solubility of uric acid in plasma and the plasma uric acid concentration at which urate deposition occurs, and thus influence the extent to which hyperuricaemia is complicated by gout.

Existing information on uric acid concentration in the blood and urine in relation to the diet has been derived mainly from studies on people consuming Western European or North American diets. It

would seem inappropriate to apply data from these studies to regions of the world where dietary protein deficiency is known to exist in large segments of the population, in view of the possible influence of dietary protein on the concentration and solubility limit of plasma uric acid suggested in the above studies.

Several human groups appear to have hyperuricaemia that cannot be attributed to dietary or other known environmental factors and therefore must be attributed to genetic determinants. For example, the natives of Pukapuka, an island in Polynesia, are not westernised, are not obese or hypertensive and consume no alcohol, which are all factors often associated with hyperuricaemia, yet 40% of males have serum uric acid concentrations greater than 70 mg/l (Wyngaarden and Kelley 1972). Other groups have a strong genetic predisposition to hyperuricaemia so that they respond quickly to dietary factors favouring hyperuricaemia.

Therefore genetic factors present in any population selected for SCP supplementation will have to be ascertained as well as the response of plasma uric acid concentration to the chosen level of supplementation determined in order to assess the potential for development of hyperuricaemia and gout.

The use of experimental animal models enables a controlled study of the effects of dietary SCP on plasma uric acid concentration to be carried out in the absence of possible genetic or environmental factors, which may influence the solubility or concentration of uric acid in the blood.

There was a significant rise in plasma uric acid concentration in female rats given diets containing a Fusarium mould, as the source of SCP, and oxonate for 21 d. The increase was found to be related to the length of time the oxonate was included in the diet, and to the level of mould fed. When the level of mould was only 150 g/kg diet, supplying about 1.2 g NA per kg diet, the plasma uric acid concentration rose by 36%, and, when 400 g mould/kg diet was fed, supplying about 3.2 g NA per kg diet, the plasma uric acid concentration was elevated by 70%, compared to casein-fed control animals. Therefore even small increases in dietary nucleic acid intake result in marked increases in plasma uric acid concentration.

Clinical conditions associated with hyperuricaemia

A. Gout

Gout is the syndrome, which results from the crystallisation of the sodium salt of uric acid in the body. Primary gout occurs when the body fluids become supersaturated with sodium urate because of a genetic defect, which results in either excessive synthesis, or diminished excretion of uric acid, or a combination of both. Primary gout is initially asymptomatic and may remain so, but in some affected individuals the disorder becomes clinically manifest by recurrent attacks of a characteristic type of acute arthritis or by renal lithiasis or both. Secondary gout is an acquired form of the disease in which hyperuricaemia occurs as a result of some other disorder.

In a person at genetic risk of primary gout, plasma uric acid concentration is normal in childhood, but rises excessively at puberty. In men the uric acid concentration frequently exceeds the solubility limit in plasma; this is about 64 mg/l. However in women the plasma uric acid concentration rarely rises beyond the solubility limit until the menopause. In the Western world the mean plasma uric acid concentration in men is about 51 mg/l and in premenopausal women is about 42 mg/l (PAG Bulletin 1975). At the menopause, however, the mean concentration in women rises and thereafter approximates that found in men. One of the factors protecting women against hyperuricaemia is a high renal clearance of uric acid, perhaps due to oestrogens (Wolfson 1949). However there was a dramatic rise in plasma uric acid concentration in studies described above in which female rats were fed diets containing Fusarium mould as the SCP. This rise in plasma uric acid concentration might have been even greater had male animals been used.

Primary gout is predominantly a disease of men, 96% of reported cases occurring in men. The usual age of onset is about 45 yrs, which therefore follows about 30 yrs of postpubertal hyperuricaemia. Primary gout is rare in women and if present, generally occurs after the menopause. Some of the best data available for the incidence of gout in men at different serum uric acid concentrations and age comes from a twelve year study of the population of Framingham, Mass., USA (Hall, Barry, Dawber and McNamara 1967). The table below, which also includes

values from another study conducted in France (Zalokar, Lellouch and Claude 1972), shows that the incidence of gout in men increases as a function of both the serum uric acid concentration and the mean age. The occurrence of gout therefore depends on the degree and duration of the hyperuricaemia.

Incidence (%) of gout in men related to serum uric acid concentration and age.

Serum Uric acid concentration (mg/l)	Mean Age	
	49 yrs	58 yrs
70-79	4.7%	16%
80-89	11.4%	25%
90-99	31.6%	90%
100 or above	47.6%	

However it is possible to have extensive tophaceous urate deposits with a plasma uric acid concentration of about 70 mg/l, whereas with higher plasma uric acid concentrations there may be no external signs of gout in some instances. Alsaker (1965) demonstrated that some of the circulating uric acid is loosely bound to plasma albumin, low density beta-lipoproteins, beta-2 macroglobulins, and an alpha 1-alpha 2-globulin (α_1-α_2-globulin). He postulated that α_1-α_2-globulin serves as a specific transport protein for uric acid and demonstrated a deficiency of this protein in 7 patients with primary gout (Alsaker 1968). It is possible that this binding of uric acid to plasma proteins may affect the renal clearance of uric acid and may also play a role in preventing the deposition of urate in the tissues.

Dietary purines appear to be relatively unimportant in determining the plasma uric acid concentration when normal diets are eaten, so the treatment of gouty patients on normal diets by reducing dietary purine intake has little effect on the plasma uric acid concentration. However the supplementing of normal diets with SCP with its characteristically high purine content could constitute a risk to people with a tendency to gout and with elevated plasma concentrations of uric acid.

B. Renal Factors

Other clinical conditions reported to be associated with hyperuricaemia are hyperuricaemic nephropathy and renal stones. The kidney is the organ most affected by gout and kidney disease is the cause of death in many patients with chronic gout (Talbott and Terplan 1960). In rats fed diets supplemented with oxonate and uric acid, as an animal model for the study of gouty nephropathy, urate crystals were found to be deposited in the kidney and there was nephritis associated with this deposition. (Stavric et al 1969; Klinenberg et al 1971; Waisman et al 1973; Bluestone et al 1973; Klinenberg et al 1973; Waisman et al 1974). In the present work in rats fed Fusarium mould, as the source of SCP, together with oxonate, kidney uric acid concentration was increased 7 fold and histological studies showed uric acid to be concentrated intratubularly in the collecting ducts of the pyramid region of the kidney. There was also an increase in kidney weight, which was too great to be explained solely by the uric acid deposits. Kidney protein and water determinations showed no differences in the hyperuricaemic animals compared to control animals and therefore, it appeared that the increase in kidney weight was attributable mainly to hypertrophy.

These considerations therefore suggest that any increase in plasma uric acid concentration might be a potential risk in predisposing individuals to clinical conditions associated with hyperuricaemia such as gout, hyperuricaemic nephropathy and renal stones.

Hyperuricaemia and Cardiovascular diseases

The epidemiological association between gout and cardiovascular disease has long been known, but attempts to demonstrate that the effect results from the higher plasma uric acid concentration affecting thrombus formation or more specifically affecting platelet function, have produced equivocal results in both epidemiological and experimental studies. It is probable that not all the contributions of blood platelets to the development of arterial vascular disease have been elucidated, but the central importance of platelets as the initial structural elements involved in the formation of arterial thrombosis is established. It is therefore not unreasonable to consider blood

platelets as a possible mediator in the association between hyperuricaemia and arterial vascular disease.

Several experimental studies have shown increased platelet activity with increased plasma uric acid concentrations. Newland (1968) found that the intravenous injection of uric acid in rats was followed by an increase in the incidence of ADP-induced platelet thrombi in the pulmonary arteries. The hypothesis was put forward that alterations in the blood concentration of uric acid influenced ADP-induced platelet thrombosis by an effect on the rate of degradation of ADP in the blood, uric acid itself being a metabolite in the degradation of the adenine nucleotides. In conflict with this hypothesis, Packham, Ardlie and Mustard (1969) showed that platelet deaggregation was not correlated with the loss of ADP in plasma. In suspensions of washed rabbit platelets, deaggregation occurred before there had been a significant amount of ADP conversion to AMP and adenosine. Also it seemed unlikely that deaggregation could be attributed to the formation of AMP and adenosine because the amounts formed in plasma at the time of deaggregation were much less than those required to inhibit ADP-induced platelet aggregation. Bluhm et al (1973) studied the in vitro action of uric acid on human platelets. They carried out microscopic determinations of the percentage of round and dendritic-type platelets, spread-type platelets and small platelet aggregates per 100 single consecutive platelets counted at different final plasma uric acid concentrations. As the concentration of uric acid was increased, the platelet surface activation and the tendency to aggregation increased. Using the same method, patients with primary gout, were found to have a pattern of relationship between plasma uric acid concentration and platelet activity similar to that found in the in vitro studies. On the other hand Bologna (1968) found a fall in ADP-induced platelet aggregation when uric acid (final concentration 150 mg/l) was added to citrated human platelet rich plasma followed by incubation at 37^{o}C for 60 mins.

However in none of the studies reported was hyperuricaemia induced by dietary means and this must be considered a more physiological basis for the study of the effects of hyperuricaemia on platelet function. Also this enables hyperuricaemia to be induced over a relatively long period of time without the complication of genetically

determined gout. This is particularly important as it has been suggested that, although there is a higher incidence of coronary heart disease in gouty subjects, when figures for clinically overt gouty subjects are removed, the association between the risk of coronary heart disease and hyperuricaemia disappears (Hall 1965). Dietary-induced hyperuricaemia in experimental animal models also enables the effect of raising the plasma uric acid concentration to be studied in the absence of possible genetic or environmental factors, which may influence uric acid binding to plasma proteins as suggested above.

There was a significant increase in platelet aggregation induced either by ADP or by thrombin in rats made hyperuricaemic by feeding for 21 d a nucleic acid-rich diet supplemented with oxonate. A high correlation existed between the plasma uric acid concentration and the; aggregation tendency. The whole blood platelet count was also increased in animals made hyperuricaemic compared to oxonate-free control animals, which might further increase the risk of aggregation in vivo. Both the platelet aggregation tendency and the plasma uric acid concentrations increased progressively with increasing levels of nucleic acid mould in the diet.

It is possible therefore to suggest that the platelet may be a prime reactant linking hyperuricaemia with the greater incidence of cardiovascular disease in primary gout. Also if rat and human platelets behave in a similar way any increase in the dietary nucleic acid content must be regarded as potentially increasing the risk of ischaemic heart disease in man. This must point towards the importance of dramatically reducing the nucleic acid content of any single-cell protein material before it is marketed for human consumption.

Further studies in which rats were made hyperuricaemic by feeding a nucleic acid-rich diet supplemented with oxonate, showed that plasma uric acid concentration was related to the time during which oxonate was included in the diet, although the increase in platelet aggregation tendency did not reach statistical significance until after 21 d of feeding oxonate. This was true for both ADP-induced and thrombin-induced aggregation. Therefore there was a time-lag between the increase in plasma uric acid concentration and the increase in aggrega-

tion tendency. The existence of this time lag was confirmed in another experiment in which oxonate was withdrawn from the diet after an initial period of 21 d during which hyperuricaemia and increased platelet aggregation tendency had been induced. Plasma uric acid concentration had fallen 5 d after withdrawal of oxonate, but the increased aggregation tendency did not decrease until after 10 d of feeding oxonate-free diet.

The life-span of rat platelets is about 4 days (Maupin 1969) and therefore the time lag between the increased plasma uric acid concentration and the increased platelet aggregation tendency considerably exceeds the platelet life-span.

In considering a possible mechanism of action of uric acid on platelet aggregation, it is of value to consider some of the properties of other agents, which affect platelet aggregation. Salzman and Levine (1971) put forward the hypothesis that agents, which inhibit platelet aggregation do so by initiating a rise in platelet 3'5' cyclic AMP (c AMP) concentration and that those agents, which cause or potentiate platelet aggregation do so by stimulating a fall in platelet c AMP concentration. Direct measurement of c AMP content of platelets has supported this hypothesis; Salzman and Neri (1969) found platelet c AMP increased by caffeine, which inhibits platelet aggregation, and reduced by adrenaline and by ADP, both of which stimulate platelet aggregation. Prostaglandin E_1, which is a potent inhibitor of platelet aggregation, has been reported to increase platelet c AMP concentration by stimulating platelet adenylate cyclase activity (Wolfe, Muenzer and Shalman 1970). Uric acid, which potentiates ADP-induced and thrombin-induced aggregation, may act therefore by inhibiting platelet c AMP production.

Adrenaline is similar to uric acid, in its effect on platelet aggregation in that it potentiates aggregation induced by either ADP of thrombin, although its action on thrombin-induced aggregation may, at least in part, represent potentiation of the ADP effect, since thrombin causes the release of ADP from platelets. However, the action of adrenaline, unlike that of uric acid, occurs within a few minutes of administration and is lost when adrenaline disappears from the circulation (Ardlie et al 1966).

Methyl Xanthines, such as caffeine, are structurally similar to uric acid and therefore might be expected to exhibit similar effects

on platelet aggregation. However unlike uric acid, methyl xanthines are inhibitory in their action on ADP-induced and thrombin-induced aggregation and their effect occurs within a few minutes of administration.

Acetyl salicylate (aspirin), unlike uric acid, is a powerful inhibitor of ADP-induced and thrombin-induced secondary aggregation and the release reaction without affecting primary aggregation. The effect of aspirin on platelet aggregation occurs within 2 hours of administration and continues for up to 6 days after its disappearance from the circulation (O'Brien, 1968). This continued effect of aspirin after its disappearance from the circulation might be thought initially to be similar to the continued effect shown to occur with uric acid. However the experiments with aspirin were performed in humans. The human platelet life-span is 7-14 days and so the effect of aspirin persists, unlike that of uric acid, only for the duration of the platelet life-span and normal function of platelets returns when a new generation of platelets emerges.

Acetyl salicylate is thought to exert its effect on platelet aggregation by inhibiting the release of prostaglandin E_2 (PGE_2) from the platelet. This inhibition has been shown to result from the blocking of the enzymatic conversion of arachidonic acid to an endoperoxide intermediate (PGR_2) which is readily converted into PGE_2. PGE_2 has a weak stimulatory effect on platelet aggregation and its precursors arachidonic acid and PGR_2 have more potent stimulatory effects. PGR_2 has been shown to be released during platelet aggregation induced by several agents including arachidonic acid, collagen, and adrenaline and appears outside the platelet before collagen-induced aggregation occurs. (Smith et al 1974). It has been suggested that platelet biosynthesis of PGR_2 and PGE_2 mediates, in part or in full, irreversible platelet aggregation and the release reaction induced by collagen, thrombin, arachidonic acid, adrenaline or ADP (Willis et al 1974). In view of PGR_2 being considered as a common mediator in the action of so many aggregating agents, it is possible that the effect of uric acid on platelet aggregation could also be mediated by an effect on PGR_2 production in platelets. Uric acid could potentiate the ability of ADP or thrombin to release PGR_2, which in turn would potentiate the effect of ADP or thrombin on aggregation. However it is not possible to suggest how this occurs or why there is a delay

in the effect of uric acid on aggregation.

The fact that the delay in the effect of uric acid on platelet aggregation exceeds the platelet life-span in rats may indicate that uric acid is not having an effect directly on circulating platelets, but more probably on a site of platelet production. The major site of platelet production is from the megakaryocytes in the bone marrow. One possibility is that it may be necessary for deposits of uric acid to accumulate in the bone marrow and reach a certain concentration before any effect on the megakaryocytes occurs. However preliminary histological studies on the bone marrow of rats fed nucleic acid-rich diets supplemented with oxonate for 21 d showed no detectable deposits of uric acid. Further studies are needed to elucidate the possible mechanisms involved.

Section B

The effect of alterations in dietary protein on platelet aggregation

There appear to be no experimental studies reported in the literature concerning the effect of the level of protein included in the diet on platelet function, although in an epidemiological study involving seven countries Keys (1970) claimed that no relationship existed between the proportion of total energy intake derived from dietary protein and the incidence of coronary heart disease.

If the plasma concentrations of the blood coagulation factors, which are all proteins, are sensitive to levels of dietary protein, it might be expected that feeding a low protein diet could result in a reduction in the concentrations of the plasma proteins fibrinogen, Hageman factor and gamma globulins, which have been shown to be necessary for normal platelet function (Bang et al 1972) and might therefore result in a reduction in platelet activity.

On the other hand plasma albumin, the concentration of which has been shown to vary with dietary protein levels, binds free fatty acids. Hoak et al (1970) have shown in vitro that an increase in the free fatty acid : albumin molar ratio resulted in the uptake of free fatty acids by platelets and an increase in platelet aggregation. A reduced protein intake could therefore lead to a reduction in the plasma albumin concentration and an increase in free fatty acid : albumin molar ratio and thus an increase in the uptake of free fatty acids by platelets. This in turn could lead to an increase in platelet aggregation.

In the present study carried out to determine the effect of low protein diets on platelet aggregation in rats (see Results - Expt. B1) ADP-induced and thrombin-induced platelet aggregation were reduced in animals given the low protein diet (50 g casein/kg) compared with the control group given 200 g casein/kg diet. However aggregation was not reduced in animals given 90 or 130 g casein/kg diet compared to the control group. Whole blood platelet count was also reduced in the group given 50 g casein/kg diet, which is in agreement with the findings of Brown (1954), who reported that in rats given low protein diets there was a reduction in platelet production in the bone marrow. It is possible that the reduced aggregation observed in animals given low-protein diets could result from a reduced concentration of the plasma proteins fibrinogen, Hageman factor and gamma globulins as

suggested above. However in the present work plasma albumin concentration was found to be reduced in those animals given the 90 and 50 g casein/kg diets compared to the control group. This would suggest that in vivo an increase in the free fatty acid : albumin molar ratio does not result in an increase in platelet aggregation. In a further study (see Results - Expt. B2) in which the time course of the effect of low-protein diets on platelet aggregation was examined, both the reduction in aggregation tendency and the whole blood platelet count were found to occur after 4 d of feeding the low-protein diet and became further reduced with duration on the low-protein diet. The mechanism by which low-protein diets reduce platelet aggregation needs further study, however it is interesting to note that the time course of the reduction in aggregation when rats were given low-protein diets was much more rapid than the time course for the increased aggregation observed in hyperuricaemic rats, which resulted from giving nucleic acid-rich diets supplemented with oxonate (see Discussion - Section A). This would suggest that different mechanisms are involved in the action of these agents on platelet aggregation.

Section C

i) The effect of alterations in dietary fat on platelet aggregation

Dietary oils and fats have long been thought to influence arterial thrombosis. However epidemiological studies have reached equivocal conclusions and the interpretation of the data is open to much criticism.

There appears to be no agreement on the effect of dietary fats on platelet function, no matter whether the fats are administered as a single oral load, by intravenous infusion, or by continuous feeding. The conflicting results cannot be ascribed to differences in fatty acid composition, but are probably due to the different methods used.

When fatty acids are added in vitro to platelet rich plasma or a suspension of washed platelets, there is enhanced platelet aggregation. However the relevance of these results to clinical thrombosis remains obscure as the fatty acids were administered in the unbound form, and it is known that fatty acids circulating in the body are bound to plasma albumin. The experiments of Hoak et al (1967) suggest that thrombosis does not result from the effect of "bound" fatty acids until the concentration is such that the two tight "binding" sites on the albumin molecule are no longer available. Goodman (1958) found that each albumin molecule has two tight binding sites for fatty acids, such that albumin effectively competes with other plasma proteins and blood cells and takes up available fatty acids until these two binding sites are completely filled. The albumin molecule has five other binding sites with a lesser affinity and a larger number with much less affinity for fatty acids. If the plasma albumin concentration were 40 g/l,saturation of the tight binding sites would occur with a plasma free fatty acid concentration of 1150 to 1200 μEq/l. Plasma free fatty acid concentrations of this level or greater have been found in a number of physiological and pathological conditions in which there is an increased frequency of thromboembolic complications (Rifkind 1966). If the free fatty acid concentration increases further the next five binding sites of albumin for fatty acids might not completely take up the fatty acids and binding sites of other plasma proteins and blood cells might compete effectively with albumin.

When this happens, fatty acids might affect the coagulation mechanism through the activation of some coagulation proteins (Connor et al 1961) and increase the uptake of free fatty acids by platelets, which is associated with enhanced platelet aggregation (Hoak et al 1970). Long chain, saturated fatty acids have the most pronounced in vitro effect on platelet aggregation culminating in the release reaction, which produces further aggregation through the release of ADP from the platelets (Shore et al 1963; Kerr et al 1965; Ardlie et al 1966; Hoak et al 1967). A possible explanation for saturated fatty acids being more potent agents of platelet aggregation than unsaturated fatty acids might be because saturated fatty acids are less rapidly bound by albumin than unsaturated fatty acids (Warner et al 1967).

In vivo evidence in support of the hypothesis that fatty acids can produce hypercoagulability and thrombosis under more physiological conditions has been provided by studies in which subcutaneous injections of adrenocorticotropin (ACTH) were given to rabbits. Animals exhibited hypercoagulability and thrombosis at 1 hr after the injection and associated with these events there were high plasma free fatty acid concentrations due to mobilization of endogenous lipid by the ACTH (Warner et al 1967).

When free fatty acids are taken up by platelets, they are metabolized or incorporated primarily into lecithin, although also into other lipids (Spector, Hoak, Warner and Fry 1970). The fact that platelets are capable of metabolizing fatty acids has promoted speculation as to the possible relationship between the effects of free fatty acids and prostaglandins. Prostaglandin E_1, which is a potent inhibitor of platelet aggregation and the release reaction, is neither contained in, nor synthesized by the platelets. However prostaglandin E_2 (PGE_2), which is a platelet constituent, has a weak stimulatory effect on platelet aggregation and PGE_2 precursors, arachidonic acid and an endoperoxide intermediate (PGR_2), have more potent stimulatory effects on platelet aggregation. Fatty acids, therefore, by functioning as the precursor molecules of the prostaglandins, in particular PGE_2, may well play an important role in the control of platelet activities in relation to their aggregation and to thrombosis.

Hyperlipidaemic produced by fat feeding seems not to exert a pronounced direct effect on platelet aggregation, in particular ADP-induced aggregation. Renaud et al (1970) found an increased suscept-

ibility to thrombin-induced aggregation of platelets from rats given diets rich in butter (380 g/kg diet) for 10 weeks, when compared to platelets from animals given diets rich in corn oil (320 g/kg diet). However the converse was observed for ADP-induced and collagen-induced aggregation. In another experiment two groups of rats were given a high butter diet (320 g/kg diet) supplemented with either oleic or stearic acids (50 g/kg diet) for 6 weeks. Thrombin-induced aggregation was greater in the platelet-rich plasma (PRP) from rats given stearic acid than in PRP from rats given oleic acid. Conversely collagen-induced aggregation was more extensive in PRP prepared from rats given oleic acid. In a third experiment butter-rich diets (380 g/kg diet) were given to rats for 5, 7 or 10 weeks. Thrombin-induced aggregation increased progressively with increasing periods on the thrombogenic diet; however after 5 weeks the extent of aggregation, calculated by the area under the tracing of platelet aggregation, was only 7% of that observed after 10 weeks, and after 7 weeks the extent of aggregation was still only 19% of that observed after 10 weeks on the high butter diet. It would therefore appear that it was only after 7 weeks on the higher butter diet that the dramatic rise in thrombin-induced aggregation occurred. McGregor (1974a) gave two groups of rats diets rich in either hydrogenated coconut oil or maize oil (400 g/kg diet) for 8 weeks and found that the maximum rate of aggregation (VAmax) and shape change (Vsmax) for ADP-induced aggregation was slower in the PRP from rats given the high hydrogenated coconut oil diet compared to the high maize oil group. When animals were given these diets for 4 weeks, similar but less striking results were obtained. In the present work (see Results - Expt. C1), in which a similar method of assessing platelet function to that used by Renaud et al (1970) and McGregor (1974a) was employed, diets containing high (200 g/kg diet) or low (50 g/kg diet) levels of either safflower oil or coconut oil were given to rats for 21 d. No differences were observed between any of the groups for ADP-induced aggregation. Hornstra (1975) also found no differences in ADP-induced aggregation nor in thrombin-induced or collagen-induced aggregation in rats given high sunflower seed oil or hydrogenated coconut oil, although no mention is made of the duration for which diets were given.

A possible reason for the discrepancies in results obtained from experiments in which platelet aggregation was measured in PRP from rats made hyperlipidaemic by dietary means, could be the differences in duration for which the high fat diets were given and the level of fat included in the diet. In both the studies of Renaud et al (1970) and McGregor (1974a), where a decreased ADP-induced aggregation was observed in rats given the thrombogenic diets compared to the non-thrombogenic groups, the fat content of the diet was 380 and 400 g/kg diet respectively and the duration of the experiment was 10 and 8 weeks respectively. In the present work, where no differences between the thrombogenic and non-thrombogenic groups in ADP-induced aggregation were observed, the fat content of the diet was only 200 g/kg diet and the duration of feeding these diets was only 21 d. However in general it would appear that the stimulatory effects on platelet aggregation of saturated fatty acids compared to unsaturated fatty acids suggested when fatty acids are added in vitro, are not confirmed when hyperlipidaemia is induced by dietary means.

The rather few experiments examining the effects of dietary fats on the formation of arterial thrombi in vivo suggest that saturated fats enhance thrombus formation, whereas unsaturated fats do not or are even antithrombotic. Hornstra (1975) by measuring the rapidity of thrombosis in aortic loops in rats (obliteration time) found that thrombotic tendency was related to both the amount and type of dietary fat; long chain saturated fatty acids with 14 or more carbon atoms being most thrombogenic. The results of plasma coagulation studies made it appear unlikely that changes in coagulation contribute to enhanced arterial thrombotic tendency on feeding a thrombogenic saturated fat diet. The filter loop technique was developed to measure thrombus formation in circulating arterial rat blood (Hornstra 1970). This technique involves continuous measurement of the blood pressure in front of and behind a microfilter connected to the extracorporally extended arterial circulation. When circulating platelet aggregates obstruct the filter, a pressure difference develops and from these changes the degree of aggregation is determined. From studies using this method Hornstra (1975) found a much higher degree of aggregation in animals given diets rich in hydrogenated coconut oil than in those animals given diets rich in sunflower-seed oil.

The conflict in results obtained from in vitro and in vivo methods used for assessing platelet function in rats fed saturated and unsaturated fats is probably because different methods assess different aspects of platelet function. Also in vivo methods tend to measure thrombus formation whereas in vitro methods specifically measure platelet function. This emphasizes the importance of standardising a technique of study when making comparisons between the effects of different dietary alterations on platelet activity.

ii) The effect of alterations in dietary carbohydrate on platelet aggregation

It has been suggested that high levels of sucrose in the diet may be an important and possibly vital factor in the development of ischaemic heart disease (Yudkin 1957; Cleave 1974), a view which is controversial. Yudkin and coworkers suggest that a possible mechanism of action of sucrose in causing coronary heart disease and diabetes might be through a rise in the circulating insulin concentration. In human volunteers given high sucrose-diets for 2 weeks, platelet adhesiveness (Wright 1941), electrophoretic mobility of platelets in the presence of ADP (Hampton and Mitchell 1969), and plasma insulin concentration were all increased in about 30% of the subjects. The suggestion was made that the persons in whom sucrose produces 'hyper-insulinism' were also those, who were susceptible to the action of sucrose in producing ischaemic heart disease (Szanto and Yudkin 1969; Yudkin, et al 1969; Yudkin 1971). This prediction was tested in two groups of subjects. The first was a group of patients with peripheral vascular disease, the second was a group of apparently healthy males from which those with signs of diabetes, or of hypertension, or of ischaemic heart disease as revealed by electrocardiology had been excluded. The sucrose intake of the two groups was similar. Both platelet adhesiveness and the plasma insulin concentration were higher in the patients with peripheral vascular disease than in the controls. Furthermore there was a significant correlation between the sugar intake and platelet adhesiveness, and plasma insulin concentration in this group. There was no such correlation in the group of healthy controls (Yudkin and Szanto 1970).

Opie (1975) suggested that a possible explanation of the 'hyper-insulinism' in some subjects eating sucrose-rich diets, but not in

others, might be a higher level of exercise in the susceptible individuals. He suggested that the amount of exercise could alter the metabolic response of the body to increased dietary sucrose and reduce the circulating blood glucose concentration for a given concentration of insulin.

Keys (1970) in an epidemiological study entitled "Coronary heart disease in seven countries" observed a significant relationship between the incidence of coronary heart disease and the proportion of total energy supplied by sucrose. However Keys also noted that there was an even more significant correlation between the incidence of coronary heart disease and the proportion of total energy supplied by dietary saturated fatty acids. The correlation between sucrose and saturated fatty acids in the diet was high and was considered to be adequate to explain the observed relationship between sucrose and coronary heart disease. Even so however experimental studies are not unanimous in supporting the relationship between coronary heart disease, as assessed by platelet aggregation tests, and saturated fatty acid intake (see Discussion - C(i)). A United Kingdom Medical Research Council (MRC) working party was set up in 1970 (Bennett, Doll and Howell 1970) in which sugar consumption of men with myocardial infarction was compared with that of matched controls in four different centres. The average sugar consumption was slightly greater in patients with myocardial infarction than among the controls, but the differences were not statistically significant. Findings in one centre suggested that the slightly higher sugar intake in patients with myocardial infarction was likely to be due to an association between the consumption of sugar and the smoking of cigarettes.

Cleave (1974) suggested that an inaccuracy in many epidemiological studies, relating carbohydrate intake to the incidence of coronary heart disease, including the studies of Keys (1970) and the MRC (Bennett et al 1970) described above, is that only sucrose intake is considered and perhaps all forms of refined carbohydrate should be considered.

In view of the lack of agreement in relating sugar intake to the incidence of coronary heart disease and the fact that in epidemiological studies so many other uncontrolled environmental factors, apart from the one in question, as well as genetic determinants are involved, the use of experimental animal models offers an ideal means of investi-

gating the effect of dietary sucrose on platelet function, a factor of major importance in thrombus formation. An experiment was therefore carried out to examine the effect of dietary sucrose on platelet aggregation in rats (see Results - Expt. C2). Rats were given diets containing high (400 g/kg diet) or low (50 g/kg diet) levels of sucrose for 21 d, maize starch being substituted in the control group. A small decrease in ADP-induced aggregation was observed in the rats given the diet containing a high level of sucrose compared to the low sucrose group. Thrombin-induced aggregation showed a small trend in the opposite direction. In another experiment high (400 g/kg diet) or low (50 g/kg diet) levels of fructose or glucose were included in the diet of rats for 21 d (see Results - Expt. C3). No differences were observed between the two types of sugar in their effect on ADP-induced or thrombin-induced aggregation. However there were small, non-significant decreases in aggregation induced by both aggregating agents in the groups given high levels of sugar compared to the low sugar groups. In a similar study McGregor (1974b) gave rats diets containing 690 g sucrose, starch or glucose/kg for 56 d. No differences were observed in the maximum rate of aggregation (VAmax) for ADP-induced aggregation, but the maximum rate of shape change (Vsmax) was significantly increased in the group given starch compared to the other groups. In a second experiment rats were given diets rich in sucrose or starch for 18 d. No differences in VAmax or Vsmax for ADP-induced or thrombin-induced aggregation were observed between the groups. When animals were meal-fed for 4 hr/d diets containing high levels of either sucrose or maize starch for 84 d, again there were no differences in platelet behaviour between the groups.

It would therefore appear that the type of sugar included in the diet does not affect platelet function as assessed by tests of platelet aggregation in rats. High dietary levels of all sugars appear to have a slight inhibitory effect on platelet aggregation. This does not support the suggestion that high levels of sucrose per se in the diet is a determining factor in the development of ischaemic heart disease via an effect on platelet function.

Section D

The effect of dietary interactions between protein, fat and carbohydrate on platelet aggregation

Very little work has been reported in the literature concerning the interaction between dietary protein, fat and carbohydrate in their effect on platelet function. To test the possible interactions between these dietary components in their effect on platelet aggregation, an experiment was set up on a 2 x 2 x 2 block factorial design (see Results - Section D). Rats were given diets containing 50 or 200 g casein, 50 or 200 g safflower oil and 50 or 400 g sucrose/kg for 21 d and ADP-induced and thrombin-induced platelet aggregation was measured. There were reductions in ADP-induced and thrombin-induced platelet aggregation and whole blood platelet count in rats given the low-protein diets compared to the high-protein groups and these effects occurred regardless of the sucrose or safflower oil content of the diets. In rats given low-protein, low-sucrose diets ADP-induced aggregation and whole blood platelet count were increased in those animals also given the diets containing a high level of safflower oil compared to the low fat group. This effect of fat was however lost when the low protein group also contained a high level of sucrose. Therefore when low protein diets are given to rats, high levels of sucrose appear to be protective against the increased aggregation, which occurs when high levels of safflower oil are included in the diet. When rats were given high protein diets no significant differences were observed in ADP-induced or thrombin-induced aggregation between the groups regardless of the sucrose or fat content of the diet, although there was a trend towards diets containing high levels of sucrose being protective against platelet aggregation when compared to high-protein diets containing low levels of sucrose. This trend compares favourably with the results from Expts C2 and 3, where high-protein, high-sugar diets produced slightly reduced aggregation compared with high-protein, low-sugar groups. No differences in kaolin-cephalin clotting time were observed between the groups, which suggests that any effects observed on platelet aggregation were due to effects on the platelets themselves rather than to effects on the plasma.

Ball, Clower, Williams and Jackson (1965) reported that mice given diets containing a high level of fat (280 g lard/kg) and a low level of protein (80 g casein/kg) for 7 weeks developed a higher incidence of atrial thrombosis than animals kept on adequate commercial diets. A striking feature of the low-protein, high-fat diet of Ball et al (1965) was a severe chronic anaemia, which was readily reversible on refeeding a normal diet. Ashburn, Weaver and Summers (1972) showed that prevention of anaemia by intraperitoneal injection of red cells also prevented the development of atrial thrombi. It was suggested that the combined local and general hypoxia due to the anaemia and endothelial oedema are the thrombogenic factors on feeding this type of diet. In the present study increased platelet aggregation was found, when diets containing low levels of protein and high levels of safflower were given, but this was only when compared with the low-protein, low-fat group; compared with the high-protein groups platelet aggregation was reduced in all the low-protein groups including the high fat, low protein group. It would appear therefore in the present study that the level of safflower oil only had an effect on platelet aggregation when diets were low in protein, and this effect was only to counteract some of the reduced aggregation, which occurred as a result of giving low protein diets.

Bibliography

Allard, C. and Goulet, C. (1973) Can. Med. Ass. J. 109 986.

Alvsaker, J.O. (1965) Scand. J. Clin. Lab. Invest. 17 476.

Alvsaker, J.O. (1968) J. Clin. Invest. 47, 1254

Ardlie, N.G., Kinlough, R.L., Glew, G. and Schwartz, C.J. (1966) Aust. J. Exp. Biol. Med. Sci. 44 105.

Ashburn, A.D., Weaver, M.M. and Summers, P.A. (1972) Amer. J. Anat. 133 341.

Ask-Upmark, E. and Adner, M.L. (1950) Acta. Med. Scand. 139 1.

Aster, R.H. (1966) J. Clin. Invest. 45 645.

Aster, R.H. (1969) Blood 34 117.

Baker, R., Blaschko, H. and Born, G.V.R. (1959) J. Physiol. (London) 149 55 p.

Ball, C.R., Clower, B.R., Williams, W.L. and Jackson, M. (1965) Arch. Path. 80 391.

Bang, N.U., Heidenreich, R.O. and Trygstad, C.W. (1972) Ann. N.Y. Acad. Sci. 201 280.

Behnhe, O. (1967) Anat. Rec. 158 121.

Bennett, A.E., Doll, R. and Howell, R.W. (1970) Lancet 1 1011.

Bennett, P.N. (1967) J. Atherosclerosis. Res. 7 53.

Berg, K.J. (1965) Lancet 2 980.

Bettex-Galland, M. and Lüscher, E.F. (1961) Biochim. Biophys. Acta. 49 536.

Biggs, R. (1972) In Human Blood Coagulation, Haemostasis and Thrombosis p.159 Ed. by R. Biggs, Blackwell Scientific Publications.

Bluestone, R., Waisman, J. and Klinenberg, J.R. (1973) Lab. Invest. 30 716.

Bluhm, G.B. and Riddle, J.M. (1973) Seminars Arthritis and Rheumatism 2 355.

Bologna, E. (1968) Boll. Soc. Ital. Sper. 45 1027 (Ital.)

Born, G.V.R. (1962) Nature (Lond.) 194 927.

Born, G.V.R. (1970) J. Physiol.(Lond.) 209 487.

Born, G.V.R. (1972) Adv. Exp. Med. Biol. 34 3.

Born, G.V.R. and Cross, M.J. (1963) J. Physiol. (Lond.) 168 178.

Born, G.V.R. and Cross, M.J. (1964) J. Physiol. (Lond.) 170 397.

Botti, R.E. and Ratnoff, O.D. (1963) J. Clin. Invest. 42 1569.

Bounameaux, Y. (1959) Compt. Rend. Soc. Biol. (Paris) 153 685.

Brandenberger, H. (1954) Biochim. Biophys. Acta. 15 108.

Bridges, J.M., Dalby, A.M., Miller, J.H. and Weaver, J.A. (1965) Lancet 1 75.

Brown, J.W. (1954) Anat. Rec. 120 515.

Calloway, D.H. (1974) In Single-cell protein. Proceedings of the International Symposium in Rome. Ed. by P. Davis.

Chandrasekhar, N. (1967) Blood 30 544.

Cleave, T.L. (1974) In The Saccharine Disease p.97-119. Bristol, John Wright and Sons Ltd.

Cucuianu,M., Papilian, W., Bareluic, N. and Crisnic, I. (1969) Thromb. Diath. Haemorrh. 22 164.

Darlington, L.G., Shaw, S. and Scott, J.T. (1971) Ann. Rheum. Dis. 30 541.

Davie, E.W. and Ratnoff, O.D. (1964) Science 145 1310.

Davis, J.W., Wilson, S.J. and McField, J.R. (1967) Blood 30 556.

Day, H.J., Holmsen, H. and Hovig, T. (1969) Scand. J. Haematol. Suppl. 7 1-35.

Day, H.J. and Holmsen, H. (1971) Ser. Haematol. 4 3.

Dosman, J.A., Crawhall, J.C. and Klassen, G.A. (1975) Metabolism 24 473.

Douglas, A.S. (1969) In Recent Advances in Blood Coagulation. Ed. by L. Poller.

Dreyfus, F. (1960) Dis. Chest. 38 332.

Duff, G.L. and McMillan, G.C. (1951) Amer. J. Med. 11 92.

Ebbe, S. (1970) In Regulation of Haematopoiesis Vol.2 p.1587-1610. Ed. by A.S. Gordon, Appleton-Century-Crofts, New York.

Ebbe, S. (1971) In Circulating Platelet p.19-43 Ed. by S.A. Johnson Academic press, New York and London.

Edozien, J.C., Udo, U.U., Young, V.R. and Scrimshaw, N.S. (1970) Nature 228 180.

Eidlitz, M. (1961) Lancet 2 1046.

Emmerson, B.T. (1974) Ann. Rheum. Dis. 33 276.

Emmons, P.R., Hampton, J.R., Harrison,M.J.G., Honour, A.J. and Mitchell, J.R.A. (1967) Brit. Med. J. 2 468.

Fleischman, A.I., Justice, D., Watson, P. and Bierenbaum, M.L. (1974) Fed. Proc. 33 244.

Fleischman, A.I., Bierenbaum, M.L., Justice, D., Stier, A., Sullivan, A. and Fleischman, M. (1975) Amer. J. Clin. Nutr. 28 601.

French, J.E., Macfarlane, R.G. and Sanders, A.G. (1964) Brit. J. Exp. Pathol. 45 467.

Fridovich, L. (1965) J. Biol. Chem. 240 (2) 2491.

Gaardaer, A., Jenson, J., Laland, S., Hellem, J. and Owren, P.A. (1961) Nature (Lond.) 192 531.

Geill, T. and Dybkaer, R. (1969) Scand. J. Clin. Lab. Invest. 23 255.

Gertler, M.M., Garn, S.M. and Levine, S.A. (1951) Ann. Int. Med. 34 1421.

Goldsmith, H.L. (1971) Fed. Proc. 30 1578.

Gomori, G. (1951) Methods in Medical Research 4 1 Ed. by M.R. Vasscher. Year Book Publishers.

Goodman, D.S. (1958) J. Amer. Chem. Soc. 80 3892.

Grayzel, A.I., Liddle, L. and Seegmiller, J.E. (1961) New. Eng. J. Med. 263 763.

Gugler, E. and Lüscher, E.F. (1965) 14 361. Thromb. Diath. Haemorrh.

Gutman, A.B. and Yu, T.F. (1957) Amer. J. Med. 23 600.

Hall, A.P. (1965) Arthritis Rheum. 8 846.

Hall, A.P., Barry, P.E., Dawber, T.R. and McNamara, P.M. (1967) Amer. J. Med. 42 27.

Hampton, J.R. and Mitchell, J.R.A. (1969) Brit. Med. J. (i) 1074.

Hansen, O.E. (1966) Am. Heart. J. 72 570.

Haslam, R.J. (1964) Nature (Lond.) 202 765.

Haslam, R.J. (1967) In Physiology of Haemostasis and Thrombosis. Ed. by S.A. Johnson and W.H. Seegers.

Hellem, A.J. (1960) Scand. J. Clin. Lab. Invest. 12 Suppl. 51.

Hellem, A.J. and Stormorken, H. (1969) In Recent Advances in Blood Coagulation. Ed. by L. Poller.

Heuchenkamp, P.U. and Zollner, N. (1971) Lancet 1 808.

Hoak, J.C., Warner, E.D. and Connor, W.E. (1967) Circ. Res. 20 11.

Hoak, J.C., Spector, A.A., Fry, G.L. and Warner, E.D. (1970) Nature 228 1330.

Holmsen, H., Day, H.J. and Stormorken, H. (1969) Scand. J. Haematol. Suppl.8 1.

Hornstra, G. (1971) Nutr. Metab. 13 140.

Hornstra, G. (1973) Haemostasis 2 21.

Hornstra, G. (1975) In 'The role of fats in human nutrition' p.303-330. Ed. by Vergroesen Academic Press.

Hornstra, G., Chait, A., Lewis, B., Turpeinen, O., Karvonen, M.J. and Vergroesen, A.J. (1973) Lancet 1 1155.

Hornstra, G. and Lussenberg, R.N. (1975) Atherosclerosis 22 499.

Hornstra, G. and Vendelmans-Starrenburg, A. (1973) Atherosclerosis 17 369.

Hugues, J. (1960) Compt. Rend. Soc. Biol. (Paris) 154 866.

Jamieson, G.A., Urban, C.L. and Barber, A.J. (1971) Nature New Biol. 234 5.

Johnson, S.A. (1971) In "Circulating Platelet p.355-93. Ed. by S.A. Johnson Academic Press New York and London.

Johnson, W.J., Stavric, B. and Chartrand, A. (1969) Proc. Soc. Exp. Biol. Med. 131 8.

Kellermeyer, R.W. and Brechenbridge, R.T. (1965) J. Lab. Clin. Med. 65 307.

Kerr, J.W., Aulay, I., Pirrie, R. and Bronte-Stewart, B. (1965) Lancet 1 1296.

Keys, A. (1976) Circulation "Coronary Heart Disease in Seven Countries" 41 Suppl. 1.

Kihlberg, R. (1972) Ann. Rev. Microbiol. 26 427.

Kim, W.M., Merskey, C., Deming, Q.B., Adel, H.N., Wolinsky, H., Clarkson, T.B. and Lofland, H.B. (1976) Blood 47 275.

Kinlough-Rathbone, R.L., Packham, M.A. and Mustard, J.F. (1970) Brit. J. of Haemat. 19 559.

Klinenberg, J.R. and Kippen, I. (1970) J. Lab. Clin. Med. 75 503.

Klinenberg, J.R., King, E. and Waisman, J. (1971) Arthritis Rheum. 14 394.

Klinenberg, J.R., Bluestone, R., Schlosstein, L., Waisman, J. and Whitehouse, M.W. (1973) Ann. Intern. Med. 78 99.

Kloeze, J. (1967) Prostaglandins, 2nd Nobel Symposium p.241-252 Bergström, S. and Samuelsson, B.

Kloeze, J. (1969) Biochim. Biophys. Acta. 187 285.

Kloeze, J. (1970) Thromb. Diath. Haemorrh. 23 286.

Kloeze, J., Houtsmuller, U.N.T. and Vles, R.O. (1969) J. Atherosclerosis Res. 9 319.

Kogut, M.D., Roe, T.F., Ng, W. and Donnell, G.N. (1975) Ped. Res. 9 774.

Köhn, P.M. and Prozan, G.B. (1959) J. Am. Med. Ass. 170 1909.

Kramer, D.W., Perilstein, P.K. and De Medeiros (1958) Angiology 9 162.

Kwaan, H.C. (1969) In "Dynamics of Thrombus Formation and Dissolution" Ed. by S.A. Johnson and M.M. Guest p.114-120 Lippincott, Philadelphia, Pennsylvania.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193 265.

Lüscher, E.F. and Bettex-Galland, M. (1971) In Circulating Platelet p.225-39 Ed. by S.A. Johnson Academic Press, New York and London.

Macfarlane, R.G. (1964) Nature (Lond.) 202 498.

Macfarlane, R.G. (1965) Thromb. Diath. Haemorrh. Suppl.17 45.

Macmillan, D.C. (1966) Nature (Lond.) 211 140.

Macmillan, D.C. and Sim, A.K. (1970) Thromb. Diath. Haemorrh. 24 385.

Macmillan, D.C. and Oliver, M.F. (1965) J. Atherosclerosis Res. 5 440.

Maenpaa, P.H., Raivio, K.O. and Kehomaki, M.P. (1968) Science 161 1253.

Marcus, A.J., Ullman, H.L. and Safier, L.B. (1969) J. Lipid Res. 10 108.

Marcus, A.J. and Zucker, M.B. (1965) In The Physiology of Blood Platelets. Grune and Stratton, New York.

Marcus, A.J., Zucker-Franklin, D., Safier, L.B. and Ullman, H.L. (1966) J. Clin. Invest. 45 14.

Matteus, J.K., Wolf, C.E., Cevallos, W.H. and Holmes, W.L. (1968) Med. Exp. 18 (1) 12.

Maupin, B. (1969) In Blood Platelets in man and animals Vol. I p. 472-520 Pergamon Press.

McGregor, L. (1974a) Proc. Nutr. Soc. 33 1A.

McGregor, L. (1974b) Proc. Nutr. Soc. 33 23A.

McPherson, V.J., Zucker, M.B., Friedberg, N.M. and Rifkin, P.L. (1974) Blood 44 411.

McPherson, V.J. and Zucker, M.B. (1976) Blood 47 55.

Michal, F. and Born, G.V.R. (1971) Nature (Lond.) 231 220.

Miller, S.A. (1968) In Single-cell protein. Ed. by R.I. Mateles and S.R. Tannenbaum. M.I.T. Press.

Mills, D.C.B. and Roberts, G.C.K. (1967) J. Physiol. (Lond.) 193 443.

Mills, D.C.B. (1970) Symposium of the Zoological Society of London No.27 p.75.

Mustard, J.F. and Packham, M.A. (1970) Pharmacol. rev. 22 97.

Mustard, J.F., Murphy, E.A., Ogryzlo, M.A. and Smythe, H.A. (1963) Can. Med. Ass. J. 89 1207.

Mustard, J.F., Glynn, M.F., Nishizawa, E.E. and Packham, M.A. (1967) Fed. Proc. 26 106.

Myers, A.R., Epstein, F.H., Dodge, H.J. and Mikkelsen, W.M. (1968) Am. J. Med. 45 520.

Newland, H. (1968) Am. J. Med. Sci. 256 44.

Niewiarowski, S. (1967) In Physiology of Haemostasis and Thrombosis p.71-73, Ed. by S.A. Johnson and W.H. Seegers, Thomas, Springfield, Illinois.

Nordöy, A. (1965) Thromb. Diath. Haemorrh. 13 543.

Nordöy, A. and Rödset, J.M. (1971) Acta. Med. Scand. 190 27.'

Norrlind, B. and Kihlberg, R. (1972) Report No.35 Tillämpad Mikrobiologi, Karolinska Institute, Stockholm.

Nugteren, D.H. and Hazelhof, E. (1973) Biochim. Biophys. Acta. 326 448.

O'Brien, J.R. (1968) Lancet 1 779.

Opie, L.H. (1975) Am. Heart. J. 89 674.

Owren, P.A. (1965a) Ann. Intern. Med. 63 167.

Owren, P.A. (1965b) Ann. Intern. Med. 63 1160.

Owren, P.A., Hellem, A.J. and Odegaard, A. (1964) Lancet 2 975.

Packham, M.A., Ardlie, N.G. and Mustard, J.F. (1969) Amer. J. Physiol. 217 1009.

Packham, M.A., Evans, G., Glynn, M.F. and Mustard, J.F. (1969) J. Lab. Clin. Med. 73 686.

PAG (1972) Bulletin Vol. II No.2.

PAG (1975) Bulletin Vol. V No.3.

Perheentupa, J. and Raivio, K. (1967) Lancet 2 528.

Peters, J.P. and Van Slyke, K.K. (1946) Quantitative chemistry Vol. I p.937 2nd edition, Williams and Williams, Baltimore.

Pinto, G.F. and Aranjo Neto S.A. (1972) Archivas Latinamer Nutricion 22 49.

Poole, J.C.F. and French, J.E. (1961) J. Atherosclero~~sis~~ Res. 1 251.

Renaud, S., Kinlough, R.L. and Mustard, J.F. (1970) Lab. Invest. 22 339.

Renaud, S. and Gautheron, P. (1974) Atherosclerosis 20-21 115.

Rifkind, B.M. (1966) J. Atherosclero~~sis~~ Res. 6 26.

Salzman, E.W. (1963) J. Lab. Clin. Med. 62 724.

Salzman, E.W. (1972) New Eng. J. Med. 286 358.

Salzman, E.W., Chambers, D.A. and Neri, L.L. (1966) Nature (Lond.) 210 167.

Salzman, E.W. and Levine, L. (1971) J. Clin. Invest. 50 131.

Salzman, E.W. and Neri, L.L. (1969) Nature (Lond.) 224 609.

Schiffman, S., Rapaport, S.I. and Chong, M.M.Y. (1966) Proc. Soc. Exp. Biol. Med. 123 736.

Scrimshaw, N.S. (1975) In Single-cell protein II Ed. by S.R. Tannenbaum and D.I.C. Wang. M.I.T. Press.

Shaper, A.G. (1974) In Cardiovascular Disease in the Tropics p.148-160 Ed. by A.G. Shaper, M.S.R. Hutt and Z. Fejfar BMA, Lavenham Press.

Shore, P.A. and Alpers, H.S. (1963) Nature (Lond.) 200 1331.

Shreiner, D.P. and Levin, J. (1973) In Haemopoietic stem cells, Ciba Foundation Symposium, 13 p.225-41.

Silver, M.J., Smith, J.B., Ingerman, C. and Kocsis, J.J. (1972) Prostaglandins 1 429.

Sinakos Z. and Caen J.P. (1967) Thromb. Diath. Haemorrh. 17 99

Smith, J.R., Ingerman, C., Kocsis, J.J. and Silver, M.J. (1973) J. Clin. Invest. 52 965.

Smith, J.B., Ingerman, C., Kocsis, J.J. and Silver, M.J. (1974) J. Clin. Invest. 53 1468.

Spaet, T.H. and Stemerman, M.B. (1972) Ann. N.Y. Acad. Sci. 201 13.

Spector, A.A., Hoak, J.C., Warner, E.D. and Fry, G.L. (1970) J. Clin. Invest. 49 1489.

Spring, M., Cavusoglu, M., Chu, Y.C. and Artymo-wshe, C. (1960) Circulation 22 817.

Stavric, B., Johnson, W.J. and Grice, H.C. (1969) Proc. Soc. Exp. Biol. Med. 130 512.

Stemerman, M.B., Baumgartner, H.R. and Spaet, T.H. (1971) Lab. Invest. 24 179.

Stirpe, F., Della Corte, E., Bonetti, E., Abbondawza, A., Abbati, A. and de Stefano (1970) Lancet 2 1310.

Szanto, S. and Yudkin, J. (1969) Postgrad. Med. 45 602.

Szanto, S. and Yudkin, J. (1970) Proc. Nutr. Soc. 29 Suppl.3A.

Talbott, J.H. (1964) Gout p.139 Grune and Stra-ton, N.Y.

Talbott, J.H. and Terplan, K.L. (1960) Medicine (Baltimore) 39 405.

Thomas, D.P. (1972) Adv. Exp. Med. Biol. 34.

Tranzer, J.P. and Baumgartner, H.R. (1967) Nature (Lond.) 216 1126.

Tranzer, J.P., Da Prada M. and Pletscher, A. (1966) Nature (Lond.) 212 574.

Udo, U.U. (1969) Masters Thesis, Dept. Nutrition and Food Science, M.I.T., Cambridge, Mass.

Vergroesen, A.J. and Gottenbos, J.J. (1975) In The role of fats in human nutrition p.1-41. Ed. by A.J. Vergroesen, Academic Press.

Verstraete, M. (1972) Acta. Cardiol. (Brux) Suppl. 15 37.

Waisman, J., Bluestone, R. and Klinenberg, J.R. (1973) Amer. J. Pathol. 70 3a.

Waisman, J., Bluestone, R. and Klinenberg, J.R. (1974) Lab. Invest. 30 716.

Walsh, P.N. (1972a) Brit. J. Haematol. 22 237.

Walsh, P.N. (1972b) Brit. J. Haematol. 22 393.

Walsh, P.N. (1974) Blood 43 597.

Walsh, P.N. and Biggs, R. (1972) Brit. J. Haematol. 22 743.

Waslien, C.I., Calloway, D.H. and Margen, S. (1968) Amer. J. Clin. Nutr. 21 892.

White, J.G. (1971) In Circulating Platelet p.46-122. Ed. by S.A. Johnson Academic Press, New York and London.

Willis, A.L. (1974a) Prostaglandins 5 1.

Willis, A.L. (1974b) Science 183 325.

Willis, A.L., Vane, F.M., Kuhn, D.C., Scott, C.G. and Petrin, M. (1974) Prostaglandins 8 (6) 453.

Willis, A.L., Comai, K., Kuhn, D.C. and Paulsrud, J. (1974) Prostaglandins 8 (6) 509.

Wolfe, S.M., Muenzer, J. and Shulman, N.R. (1970) J. Clin. Invest. 49 104.

Wolfson, W.Q., Hunt, H.D., Levine, R., Guterman, H.S., Cohn, C., Rosenberg, E.F., Huddleston, B. and Kadota, K. (1949) J. Clin. Endocr. 9 749.

Wright, H.P. (1941) J. Path. Bact. 53 255.

Wright, J.H. (1906) Boston Med. Surg. J. 154 643.

Wyngaarden, J.B. and Kelley, W.N. (1772) In The metabolic basis of inherited disease, p.667-728 Ed. by J.B. Stanbury, J.B. Wyngaarden and D.S. Fredrickson, McGraw Hill, N.Y. and London.

Yudkin, J. (1957) Lancet 2 155.

Yudkin, J. (1971) In Sugar p.231-42, Ed. by J. Yudkin, J. Edelman and L. Hough, London Butterworths.

Yudkin, J. (1972) Nature (Lond.) 239 197.

Yudkin, J. and Roddy, J. (1964) Lancet 2 6.

Yudkin, J. and Szanto, S. (1970) Proc. Nutr. Soc. 29 Suppl.3A.

Yudkin, J., Szanto, S. and Kakkar, V.V. (1969) Postgrad. Med. J. 45 608.

Zalokar, J., Lellouch, J. and Claude, J.R. (1972) J. Chron. Dis. 25 305.

Zucker, M.B., Rifkin, P.L., Friedberg, N.M. and Coller, B.S. (1972) Ann. N.Y. Acad. Sci. 201 138.

Zucker, M.B. and Vroman, L. (1969) Proc. Soc. Exp. Biol. Med. 131 318.