FATTY-ACID RATIOS IN FREE-LIVING AND DOMESTIC ANIMALS

Possible Implications for Atheroma

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Summary A comparison has been made of the tissue fatty acids in domestic bovids and bovids from free-living and undisturbed habitats. In the domestic state, the proportion of polyunsaturated to non-essential fatty acids was of the order of 1/50, whereas in the free-living animal it was 1/2·3. There also seemed to be a greater diversity of polyunsaturated acids in free-living species. These differences may arise partly because oil-rich vegetation which is available to free-living animals has been eliminated from the diet of domestic animals raised on grassland. Since man's tissue lipids approximate to the domestic pattern on which he is dependent, the question arises as to whether the total domestic development of water-rich vegetation is nutritionally detrimental and that a resultant low balance of polyunsaturated to saturated and monounsaturated fatty acids may be related to arterial disease.

Introduction

THE amount and type of dietary fat may be related to atheroma (Ahrens et al. 1958, Funch et al. 1960). Death from arterial disease is correlated with a high intake of animal fat, protein, and calories (Yerushalmy and Hilleboe 1957) and it is likely that the animal fats and the calories are more relevant than the protein (Katz et al. 1958, Keys 1963). That the nature of the fat may be important is suggested by the fact that polyunsaturated fatty acids can protect the artery from damage in experimental situations (Thomas and Hartroft 1959, O'Neal et al. 1959, Wissler et al. 1962, Moore and Kon 1963, Kim et al. 1965).

The work on experimental atheroma tends to implicate, in some way, animal fat or carbohydrate (Sandler and Bourne 1963, Roberts and Strauss 1965). In this context, I have previously asked if, by examining data from a prosperous Western community, we may be dealing with an abnormal state (Crawford 1964, 1967).

Greaves and Tan (1966) studied dietary aminoacid profiles which in the U.K. closely resembled the pattern recommended by the Food and Agriculture Organisation; the excellence of the agreement was mainly due to the predominance of milk, and other animal products. No such recommended pattern exists for the fatty acids which are known to be essential for membrane synthesis and are diet dependent (Kingsbury et al. 1962, Bergstrom et al. 1964, Thompsen and Jakobsen 1966, van Golde and van Deenen 1966). Aitken and Hytten (1960) suggest that human (Western) milk may be insufficient with respect to essential fat. Kon (1962) suggested that the polyunsaturated-fatty-acid/energy ratio may be related to atheroma, and Sinclair (1964, 1968) argues for a role of essential-fatty-acid deficiency in atherosclerosis.

In an attempt to establish a baseline for the essential fatty acids, " natural " systems are being compared with domestic, and I present evidence here that food selection in undisturbed systems results in a balance of polyunsaturated to monounsaturated and saturated fatty acids that is sufficiently different from the end-products of the

man-made domestic system to warrant discussion of possible nutritional implications.

Materials and Methods

Animal Tissues

Hind-leg muscle tissue was obtained from wild animals through the cooperation of the Uganda Game Department and the Uganda National Parks. The African bovids (the Cape Buffalo, *Syncerus caffer*) were sampled mainly from two different environments during the dry season. Firstly, from parkland which is predominantly grassland with mixed bush and occasional trees typically seen in the plains of Ishasha, and Queen Elizabeth National Parks (Laws and Parker 1968). Secondly, free-living from woodland and bush country as seen in the forests of Ishasha, Tonia/Kaiso (near Bogoma Forest Reserve), and Para Escarpment around Budongo Forest and Atura. Animals in this situation have access to both grass and woody vegetation.

Other wild animals were sampled from the same areas but at different times of the year. All animals were mature adults.

1 lb. (450 g.) samples of meat from domestic stock were obtained through the courtesy of the local slaughterhouse and purchased from local butchers; attached adipose tissue was removed. This massive fat-deposition does not occur in wild species in which samples of at least 1 kg. (2·2 lb.) were used for total lipid content. For detailed gas-chromatographic analysis, the muscle of domestic meat was freed as far as possible from adipose tissue in the fascial planes to make the samples more comparable with the wild species.

TABLE I—LIPID CONTENT OF MEAT SAMPLES FROM WILD AND DOMESTIC ANIMALS

Source	No.	% lipid content (net weight) (mean ±S.E.M.)
Wild buffalo	14	2·8 (±0·24)
Domestic cattle (butcher's meat as sold in U.K.)	15	25·0 (±7·22)
Muscle tissue after removal of all visible fat	8	7·9 (±1·6)

Using data from table II a 1000 kg carcass from the domestic cattle will contain 50 g. of polyunsaturated fatty acids (P.U.F.A.) based on a 25% carcass fat (Ledger 1968). The buffalo would contain 80 g. per 1000 kg. based on a 3–5% total carcass fat (Ledger 1968). If lean meat only is sampled the fat-content of domestic meat approaches the 3–8% of veal (McCance and Widdowson 1962) and 1000 kg. of muscle cells would be associated with 16 g. P.U.F.A., about a fifth of that associated with the buffalo muscle cells.

African Vegetation

The vegetation used by the Hadza in East Africa was supplied by Dr. J. Woodburn. Use has also been made of data collected in West Africa by Busson (1965).

Lipid Analysis

The extraction and analysis of lipids was carried out by standard procedures (Leat 1963). Methyl esters were separated by thin-layer chromatography for preliminary analysis (Morris 1962). The results reported here are derived from quantitative gas-chromatographic analysis (Horning et al. 1964) using a ' Model 64 ' (Pye) instrument of the 104 series. Methyl esters were identified by relative retention-times on polydiethyleneglycol succinate, polyethyleneglycol adipate (P.E.G.A.), and Apiezon L columns. Bromination was used to confirm the presence of the unsaturated compounds.

In this paper the group of saturated and mono-unsaturated acids refers to those acids identified as myristic, palmitic, palmitoleic, stearic, and oleic acids. The polyunsaturated group refers to linoleic, linolenic, arachidonic, and an acid tentatively identified as C22:5 and C22:6.

Results

Table I shows that the total amounts of lipid extracted from muscle samples of domestic bovids was on the average about ten times as much as the wild. There was a wide variation in the samples from domestic stock.

TABLE II—PROPORTION OF THE POLYUNSATURATED FATTY ACIDS IN MUSCLE TISSUE LIPIDS OF FREE-LIVING AND DOMESTIC BOVIDS

Source	No.	% Poly-unsaturated (1) (mean ±S.E.M.)	% Saturated and mono-unsaturated (2) (mean ±S.E.M.)	(1)/(2)
Domestic beef	12	2·2 (±0·3)	98·8 (±0·3)	1/45
All buffalo to date	12	21·9 (±2·8)	78·7 (±2·4)	1/3·6
" Parks " buffalo	5	10 (±1·3)	90 (±2·2)	1/9
Woodland buffalo	7	30 (±1·3)	71 (±2·8)	1/2·3

When a fatty-acid profile of the order of the domestic bovid is used in the diet, 1 polyunsaturated molecule is accompanied by 45 saturated or mono-unsaturated molecules; inactive isomers may further reduce the effectiveness of the essential fatty acids. In the natural enviroment the buffalo is achieving a balance of 1 to 2-3; parkland (mixed grass and bushland) produced intermediate levels.

The proportions of polyunsaturated compared with monounsaturated and saturated fatty acids in the domestic and wild bovids are shown in table II. Approximately 30% of the fatty acids were polyunsaturated in the buffalo living in the " natural " woodland environments, and about 10% in the parks or predominantly grassland buffalo. The lipids of the domestic bovids for the U.K. had only 2% of their fatty acids in the polyunsaturated group. A detailed analysis comparing a woodland buffalo with a parks buffalo and the domestic bovids is shown in fig. 1.

Table III gives a variety of examples of individual species from both wild and domestic environments. Among the wild species, the plains or grassland animals (topi and Uganda kob) had proportions similar to those found in domestic species. The wild species in captivity showed a lipid composition of the same order as that of the domestic species.

The domestic pig had a higher proportion (8%) of unsaturated fatty acids, which may be related to the use of fish meal in the rations, and the fact that they are non-ruminants. Chicken muscle contained even higher proportions of the polyunsaturated fatty acids (17%) which again may be due to the use of grain and fish products in the rations.

Analysis of the detailed profile of the fatty acids shows

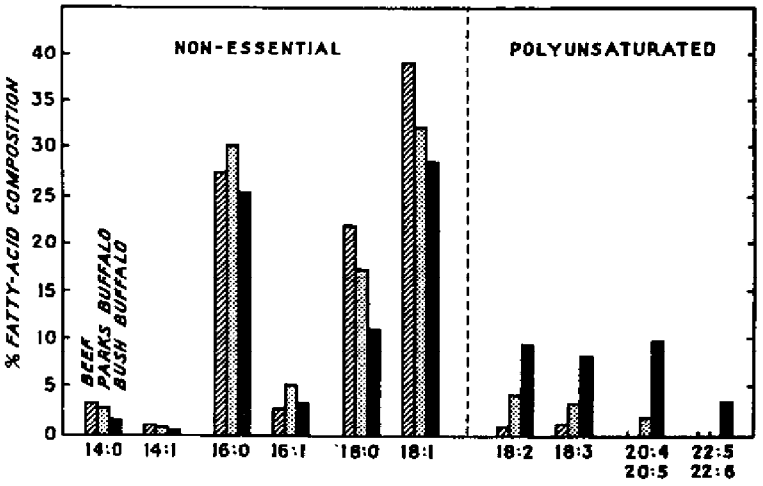

Fig. 1—Distribution of the fatty acids in muscle tissues of the woodland and parks buffalo compared with domestic cattle.

Not only is there a greater proportion of polyunsaturated fatty acids in the woodland animal but there is also a greater degree of diversity.

TABLE III—PROPORTIONS (*molecular equivalents*) OF POLYUNSATURATED FATTY ACIDS TO THE SATURATED AND MONOUNSATURATED FROM VARIOUS SOURCES

Source	Proportion	Source	Proportion
Wild African woodland/bushland:		*Captive and domestic:*	
Eland	35/65	Giraffe (mean of 2)	4/96
Hartebeest (1)	32/68	Somali fat-tailed sheep	4/96
Hartebeest (2)	29/71	Domestic pork	8/92
Topi	23/76	Man (Western)	4/96
Warthog	27/63	Beef	2/98
Giraffe	39/61	*Food sources (cattle products):*	
Plains, grassland:		Colostral milk †	3/97
Topi	8/92	Mature milk †	2/98
Uganda kob	2/98	Butter †	2/98
Birds:		*Human milk:*	
Wild grouse *	60/40	U.S.A.†	8/92
Domestic chicken	17/83	U.S.A.‡	11/89
		Japan §	20/80
		Japan §	25/75

* Hubbard and Pocklington (1968). † National Research Council (1966). ‡ Insull and Ahrens (1959). § Saito et al. (1965).

In West Africa (Busson 1965) the mean proportion in twenty-seven species of vegetation used by man is 40/60: in East Africa the proportion in four species used by the Hadza is 45/55.

The fats in the water-rich vegetables used in Western communities are seldom analysed because the total lipid is small. Examples of proportions in oil-rich materials in use are almonds (45/46), beech nut (38/62), peanut (28/72), maize (43/57), perilla (70/30), sesame (43/57), walnut (66/34), wheat germ (43/57).

The data in the above table refers to individual specimens except where a reference is cited. The proportion in the human sample is in the same order as reported by Kingsbury et al. (1962).

that there are two significant features: (1) increase in diversity of the polyunsaturated acids present in significant amounts (fig. 1) in free-living species, and (2) a reduction in the amount of oleic acid in the presence of high (i.e., "normal"?) proportions of linoleic acid (table IV). Only the major differences are presented here; a detailed chemical analysis which will include the minor components and trace amounts will be presented elsewhere.

Discussion

There is no significant difference in the muscle-protein-aminoacid composition from free-living, as compared with domestic, animals (Crawford 1968). This paper shows that there is a striking difference in the tissue lipids.

The total fat of the domestic bovid is greater than that associated with the muscle of the free-living bovids (table I); the fatty-acid composition of the tissues is also different. The muscle-tissue lipids from free-living animals contained a high proportion of polyunsaturated fatty acids of the order of 30% of the total, as compared with 2% for the domestic bovids (tables II and III). Not only was the proportion of polyunsaturated acids greater in the free-living species but there was also a greater diversity of fatty acids (fig. 1).

Whilst there is a greater degree of fat-deposition in the domestic animals (table I), the bulk of this fat is subcutaneous. In the free-living species studied, the carcass fat is low and subcutaneous fat is negligible (Ledger 1968); it seems that the total amount of polyunsaturated fatty acids in direct association with muscle cells may be greater in the free-living bovids from bush and woodland environments.

TABLE IV—PERCENTAGE COMPOSITION OF OLEIC (18:1) AND LINOLEIC (18:2) ACIDS IN DOMESTIC AND "NATURAL" RUMINANTS

Source	No.	Oleic (%) (mean ±S.E.M.)	Linoleic (%) (mean ±S.E.M.)	Ratio (oleic/linoleic)
Natural woodland environment (linoleic > 15%)	11	20 (±1·3)	19 (±1·6)	1·1
Domestic cattle	11	39 (±2·1)	0·9 (±0·1)	43·0

The free-living ruminants with a linoleic-acid proportion of greater than 15% are compared with the domestic cattle. The total of oleic and linoleic is similar in both cases but the linoleic in the free-living species is 20 times greater than in the domestic. Greer and Malcom (1965) showed that the fatty streaks in the aorta contain a higher proportion of the monounsaturated acids including oleic than the normal intima; in our experience a high tissue proportion of linoleic was accompanied by a lower proportion of oleic.

The fact that the free-living animals from a diverse woodland habitat had greater proportions of polyunsaturated fatty acids in muscle tissue (30%) compared with the same species on parkland (10%) or what is predominantly grassland (Laws and Parker 1968), and the fact that wild species in captivity fed on hay (table III) had low proportions of polyunsaturated fatty acids similar to that of the domestic species, suggests that the primary reason for the difference is dietary and is likely to be related to the use of oil-rich vegetation—such as seeds, nuts, leaves, and woody material (Busson 1965, Laws and Parker 1968) as compared with soft grasses of the domestic context. We do not know if the rumen treats oil-rich vegetation in the same way as it does the water-rich grasses.

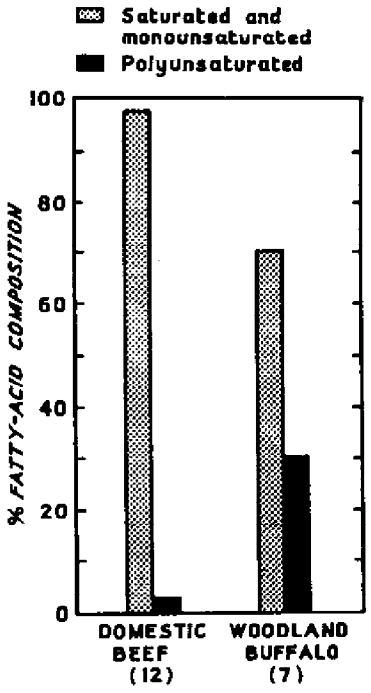

Fig. 2—Gross composition of free-living woodland bovids in Africa is compared with domestic animals.

These woodland environments can be described as relatively "undisturbed" habitats since the grasslands usually result from a combination of interference from man and animal and fire. Climax vegetation (the balance where there is no interference) in Africa or Europe is considered to be of the woodland type as opposed to grassland (Laws and Parker 1968).

Over the centuries man has developed the grassland to a high degree but it is reasonable to claim that the diverse or free-range setting of the woodland environments may be more in keeping with what can be considered to be a biologically balanced state. Whether or not the difference between the tissue fatty acids of the free-living animals and the domestic animals affects these relatively short-lived species has yet to be determined. However, the question should be raised as to whether or not the low proportions in the domestic stock might be important to the nutrition of a long-lived species, such as man, who uses their products in his diet (Greaves and Tan 1966).

Nutritional Implications

The low proportions of polyunsaturated fatty acids in meat, milk, and butter from domestic grassland bovids has been accepted as normal for some time. Other fats used by Western communities are frequently hydrogenated for use in shortenings and margarines which reduces the degree of unsaturation and produces some 20–40% unnatural isomers in the residual unsaturated

TABLE V—RELATIVE PERCENTAGE PROPORTION (%) OF FATTY ACIDS EXTRACTED FROM DIFFERENT TISSUES

Animal tissue	Position of methyl/esters: C 14 myristic	C 16 palmitic	C 16:1 palmitoleic	C 18 stearic	C 18:1 oleic	C 18:2 linoleic	C 18:3 linolenic	C 20:4 arachidonic
Human:								
Muscle ..	4	20	9	8	50	6	0·5	0·5
Heart ..	4	26	5	10	47	6	0·5	0·5
Aorta ..	2	24	7	6	48	10	0·9	2
Warthog:								
Muscle ..	Tr	18	Tr	17	19	34	6	4
Heart ..	Tr	16	Tr	19	16	35	6	6
Aorta ..	Tr	17	Tr	16	34	11	16	5
Buffalo:								
Muscle ..	Tr	20	Tr	19	26	20	7	7
Heart ..	Tr	10	Tr	26	16	30	6	10
Aorta ..	Tr	25	Tr	17	21	10	14	11

Tr = trace

The relative proportions are given of the principle monounsaturated and saturated fatty acids (appearing at > 2%) and the main polyunsaturated acids but excluding the C22 acids. The table shows that the high degree of unsaturation is extended to aorta and heart in free-living species. Preliminary work suggests different tissues may contain different proportions of the individual polyunsaturated acids. Significant amounts of the icosatrienoic acids were not detected in the aortas of the free-living animals although one C20:3 acid has been reported in human aorta tissue from Western people (Smith 1962, Greer and Malcom 1965, Scott et al. 1966) and another in fat-deficient rats (Mead and Slaton 1956).

molecules (Ostwald 1961, Aaes-Jørgensen 1966). Of the 94 vegetables listed by McCance and Widdowson (1962) as used in the U.K. only those that have been fried contain more than trace amounts of fat. Consequently, with the exception of oily fish, nuts, and the use of bottled vegetable oils, much of the food used in Western communities will contain a low proportion of polyunsaturated fatty acids.

That the diet will affect the internal composition of the fatty acids in man is suggested by the difference between the milk of American mothers which contains 8–11% polyunsaturated fatty acids and milk from Japanese mothers which contains 20–25% (Saito et al. 1965). The reliance of Western communities on a low proportion of polyunsaturated fatty acids is further suggested by the low proportions (approximately 8%) in muscle tissue (Kingsbury et al. 1962) and the almost fingerprint likeness of the fatty-acid chromatogram of man and hay-fed giraffe (table III, fig. 3).

Preliminary studies of the heart and aortic tissue patterns of the fatty acids indicate that in the free-living species the degree of polyunsaturation is extended to these tissues (table v).

Since man's evolution took place in a diverse context, the question arises as to whether or not the differences in the high proportions of polyunsaturated fatty acids available, both in the tissues of free-living species and in the vegetation, has any meaning when compared with the low proportions that form the basis of Western man's fat intake. Atheromatous lesions and cardiovascular disease is relatively common in Western communities and the possible relationship of the saturated fatty acids has been discussed by Keys (1967) and the essential fatty acids by Sinclair (1964, 1968).

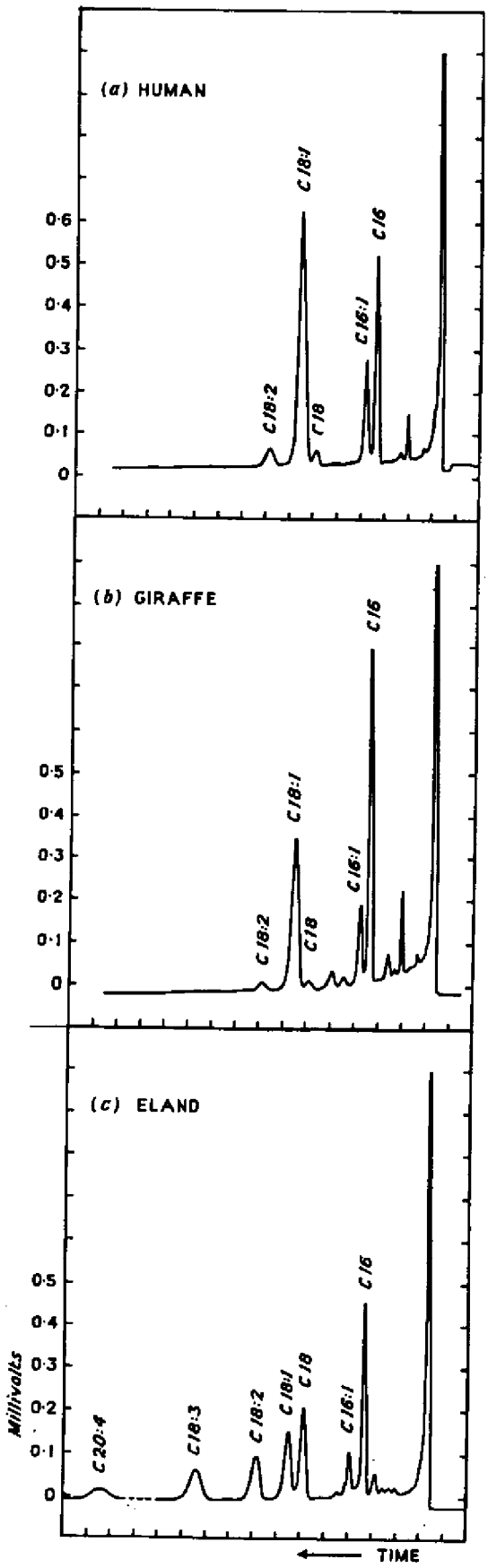

Fig. 3—Gas chromatograms show fatty-acid composition in:
(*a*) Man (Western).
(*b*) Zoo, hay-fed giraffe.
(*c*) Eland (a large African Ruminant).

The profiles for the Zoo, hay-fed giraffe and for "Western man" are very similar. This may be indicative of chronic equilibration that is difficult to examine in animal or human experiments over a few months. Chromatogram on P.E.G.A.

Sikes (1968a and b) reported that thirteen elephants living in a degenerate grassland (Laws and Parker 1968) had a high incidence of atheroma and calcium deposits in the aorta (44·5% of the aorta contained calcium deposits in the oldest category) whereas there was less (5·1%) in eighteen elephants from a scrubland habitat, and negligible amounts from nine elephants in a montane, woodland habitat. McCullagh and Lewis (1967) have confirmed the high incidence of atheroma in grassland elephants and also comment on its analogy with human atheroma.

In the findings reported here, the difference between the lipid composition in the free-living state is not just the difference between 30% and 2% but the proportion of polyunsaturated molecules to monounsaturated and saturated, which is of the order of 1/2 in natural vegetation and 1/3 available in the tissues of the free-living animals (tables II and III). In the dairy products of the domestic species used in man's diet the order is 1/50.

In other words, the difference between what is available in the natural and domestic states may involve a difference in balance of the fatty acids as well as the quantity. In similar nutritional fields the balance of components seems to be more important than the total amounts; for example, in kwashiorkor a disturbance of the essential to non-essential aminoacids may be more important than the total amount of protein (Dean and Durgess 1962, Whitehead 1964, 1965).

This change in balance from the vegetation (Busson 1965) and the animal tissues of the undisturbed habitat has presumably happened because of man's increased use of grassland and water-rich vegetation in recent centuries. At the same time, edible oil-rich vegetation and foods have been gradually eliminated. If the constituents of the oil-rich vegetation, including the polyunsaturated fatty acids, are essential for long-lived species such as man, this study could explain how the transition to the domestic state has altered the balance between water-rich and oil-rich foods in a manner which might be biologically important.

I thank my colleagues who have cooperated in this study, including Miss M. Gale, Mr. N. M. Casperd, Dr. J. Woodburn, Mrs. S. M. Crawford, my laboratory and technical staff and, especially for their assistance in preparing fig. 1 during a similar study of beef lipids (not yet published), Mr. A. W. Hubbard and Mr. W. D. Pocklington; the Game Departments of Uganda and Tanzania, and the Zoological Society of London for their cooperation; and Mr. B. Hawley for bringing the Japanese work on human milk to my attention. The work has been supported by grant no. R 1780 of the Ministry of Overseas Development and financial assistance has also been made available by the British Empire Campaign for Cancer Research, the Wellcome Trust, H. J. Heinz and Company, and the Wander Group.

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STEATORRHŒA WITH STRIKING INCREASE OF PLASMA-ALKALINE-PHOSPHATASE OF INTESTINAL ORIGIN

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Summary A patient with severe steatorrhœa of unknown cause was found to have a striking increase in plasma-alkaline-phosphatase concentration, which could not be explained by bone or liver disease. The alkaline phosphatase was shown to be largely of intestinal origin.

Introduction

NORMAL plasma may contain two distinct alkaline phosphatase (A.P.) components when examined by starch-gel electrophoresis at pH 8·6. The faster-moving component is always present and has properties similar to those of A.P. derived from liver and from bone. The slower-moving component is much more variable in amount, both between different people, and at different times in the same person. It is seen more often in people of blood-groups O and B than group A (Arfors et al. 1963) and is very seldom present in ABH non-secretors (Bamford et al. 1965). The amount present in plasma is increased after a fatty meal (Langman et al. 1966). This slow-moving component shows the properties of the main A.P. component present in extracts of mucosa from the

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