mined. The infectivity of all three types steadily declined to less than 1% of the maximal titer after 30 days. The HA of types 1 and 2 remained unchanged over a period of 28 days; type 3 HA increased over this time interval and titers of 128 HA units were reached. The infectious and noninfectious HA moiety of the periodic harvests were separated in a CsCl gradient. The changes (type 3) or stability of HA (types 1 and 2) were equally reflected in both moieties; no accumulation of noninfectious HA was found which would account for the persistence of HA and concurrent loss of infectivity.

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Intestinal Phosphatase and Fat Absorption* (33421)

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Alkaline phosphatase of intestinal origin has been identified by electrophoresis in the serum of some subjects and not in others (1–4). This component has a slower mobility in starch gel at pH 8.6 than the liver and bone fractions which constitute the remainder of the activity of normal serum. Geneticists have called attention to this polymorphism by claiming an association of blood groups O and B and the ABH secretor status in saliva with the presence of this particular isoenzyme of alkaline phosphatase in the serum (1–5). Most of these studies were carried out on serum collected without regard for meals. Recent work by Langman *et al.* (6) indicates that this relationship is important. They found that certain subjects lacking the intestinal component in the fasting state will develop it several hours after ingesting a meal with a high fat content. They reported that this occurred only in subjects who secreted blood group substances in their saliva; regularly in those of blood groups O but B only occasionally in those of blood group A. In a previous report by Warnock (7), it was found that the intestinal component could be produced in subjects lacking it in the fasting state. Blood type and secretor status were not reported in that study. Therefore the present study was carried out on subjects who were of blood type A or who were nonsecretors. Again, in every case, the intestinal component could be detected electrophoretically in the serum 3 and 5 hr after ingestion of 56 g of butter fat as cream. These studies indicate that although quantitative differences between secretors and non-secretors may be found, these differences are not qualitative. This distinction is important as a basis for understanding the significance of the rise in the serum and its relationship to fat absorption.

Material and Methods. Fourteen ambulatory medical students, physicians, and hospital personnel, aged 20–35, were selected on the

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basis of absence of an intestinal component after an overnight fast. Only subjects who were blood type A or non-secretors of ABH substance were chosen. The secretor status was determined on saliva (8) using diluted anti-A serum for subjects of blood type A and concentrated anti-H lectin (Hyland Laboratories, Los Angeles, Calif.) for subjects of blood type O. Vertical starch gel electrophoresis at pH 8.7 was performed at 4° using a Tris–borate buffer system (9). The procedure was modified from that given in that a measured 20 μ of undiluted serum was inserted in each slot. The staining solution was left on the gel for 24 hr at 4°. Although in every case 3–4 hr of staining was sufficient to detect the presence of the intestinal band, the 24-hr period was used to be sure that no intestinal component was present in the fasting state.

The selected fasting subjects drank 1 pint of commercially available cream (stated content of fat, 56 g) in the morning after a sample of venous blood was withdrawn. No further food was taken until after blood samples were withdrawn 3 and 5 hr later. Two control subjects drank 1 pint of skim milk instead of cream. In an additional experiment, 10 hospitalized patients or outpatients undergoing a glucose tolerance test were examined. These subjects were selected because of the absence of the intestinal component in the fasting blood sample. Blood types and secretor status were not determined. These subjects drank a solution containing 100 g of glucose. Blood samples were taken at 0.5, 1, 2, and 3 hr.

An estimate of the degree of sensitivity attained by the electrophoretic method in terms of units of phosphatase detectable was determined by the following procedure. Butanol extracts (10) of washed intestinal mucosa, liver, and periostum were made from tissue which appeared normal on gross examination at autopsy. The extracts were dialyzed against deionized, distilled water overnight at 4°. Quantitative analyses were performed according to Bowers (11) using *p*-nitrophenyl phosphate as substrate. Multiple doubling dilutions of the sample were made and subjected to electrophoresis in the same manner as that described above. Staining was carried out for 24 hr. The end point recorded was the number of milliunits/milliliter in the last slot showing visible staining.

TABLE I. Sensitivity of Starch Gel Method.

Tissue	Phosphatase detected (av value; mU/ml)	Range
Liver (6)[a]	1.9	1.4–3.7
Small intestinal mucosa (5)	2.2	0.8–3.2
Periosteum (6)	4.3	3.4–6.9

[a] Number of specimens tested.

Results. The results of the determination of the sensitivity of the starch gel method are given in Table I. In the case of liver, small intestinal mucosa and bone, a single major band of alkaline phosphatase is present. The kidney is not suitable for this type of dilution study because at least two major zones of activity are present (9). The gel assay will detect as little as 2–3mU/ml. Normal serum values using the same quantitative assay range from 6–110 mU/ml (11).

Table II shows that 7 group A secretors and 7 non-secretors each developed an intestinal component which was identifiable in serum samples taken at both 3 and 5 hr after ingestion of the cream (Fig. 1). Quantitative measurements of phosphatase activity in the serum yielded no consistent change in phosphatase activity. Since the gel will detect 2–3 mU/ml, the enzyme activity added to the serum in these subjects is at least that much. In one instance dilution of the sample with

TABLE II. Status of Intestinal Component after Meal.

Food	Subjects	Present	Absent
Fat:	Secretors		
	A	7	0
	Non-secretors		
	A	5	0
	O	1	0
	AB	1	0
Total		14	0
Protein: Skim milk		0	2
Carbohydrate: Glucose		0	10

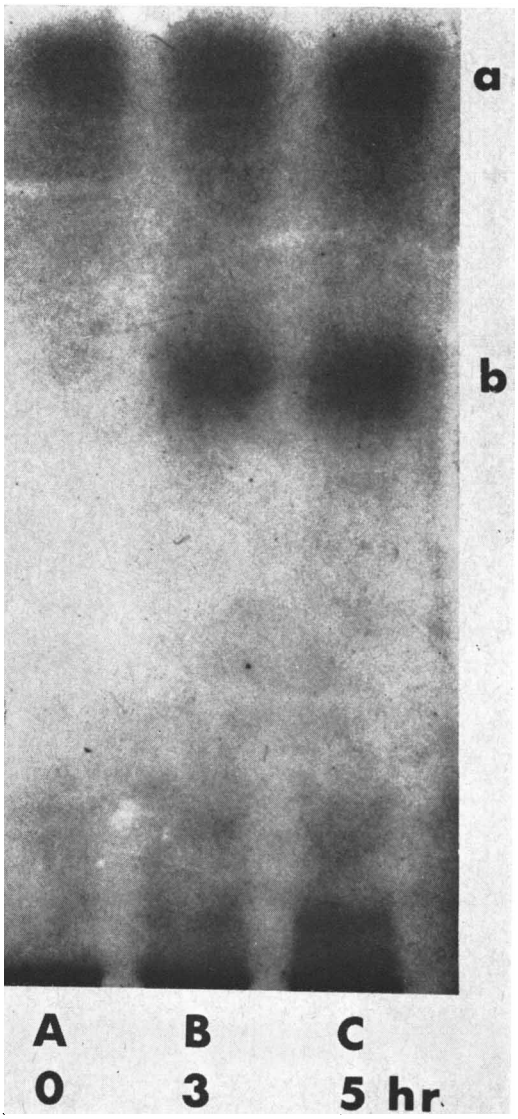

FIG. 1. Starch gel electrophoretic pattern of serum from a subject who is a type A secretor. The cathode and origin are at the bottom and the anode at the top. Left to right: (A), Fasting sample with liver component (a); (B and C), samples taken 3 and 5 hr, respectively, after ingestion of 1 pint of cream. Note development of intestinal component (b).

an equal volume of water still yielded a visible band indicating the addition of at least 4–6 mU/ml. The 2 subjects ingesting skim milk developed no intestinal isoenzyme nor did the 10 subjects undergoing glucose tolerance tests.

Discussion. The effect of diet on the presence or absence of human intestinal alkaline phosphatase has been recognized only recently (6, 12, 7), although an increase in serum alkaline phosphatase following a fatty meal was reported earlier (13). Previous studies have purported to demonstrate an association of certain blood groups with the presence of the intestinal phosphatase in the serum (1, 2). These studies make no mention of the dietary status. A comparison of 2 reports by authors using the same techniques may give some indication of the magnitude of this factor. In the earlier report in which subjects were classified without regard to the fasting state, Bamford and his associates (3) indicated that only 17% of O and B secretors have no intestinal component. Langman *et al.* (6) later found that 60% of fasted type O and B secretors lack the band. When diet was not considered 78 of 97 (80%) type A secretors and 91 of 96 (95%) non-secretors of all types had no intestinal component (3). In fasted subjects on the other hand, no intestinal component was found in 31 subjects who were type A or non-secretors (6). It is possible that the 2 populations described differ and that the above comparisons are not valid; but if they are comparable, the data certainly suggest that the postulated associations may be altered considerably by attention to diet. The authors make no mention of this discrepancy between the two reports.

Another important factor which may influence the results of studies of intestinal alkaline phosphatase is the sensitivity of the method. Starch gel electrophoresis may vary considerably in its sensitivity in different laboratories because of variations in techniques employed. Because of the sensitivity of the methods used in the present study, the results conflict with those reported elsewhere (6). Probably the most important variables are the buffer used to make the gel and the conditions employed during staining. The buffers used in the present study give good separations with undiluted serum specimens. Bamford and his associates (3) diluted their sera 1 in 3 but state that if serum without the intestinal component was "run undiluted

a very weak slow band could often be detected." They go on to state that, "This was not, however, used for classification, because the electrophoretic separation was inferior to that obtained with diluted specimens." Staining time is another variable. In several previous studies staining was carried out for only 1 hr at 37° (1–6). It is quite possible that weak bands would be missed at that time. It might also be noted that in studies in which the end point of a relatively insensitive procedure such as starch gel staining is compared with a sensitive serologic blood typing procedure, subjective factors are apt to influence the results (14).

In selecting subjects for the present study, 2 type A subjects were rejected because of presence of the intestinal component in the fasting state whereas no non-secretor was rejected. It seems probable that prolonged fasting would lead to disappearance of the intestinal component in all subjects. Dietary factors may account for the lack of the intestinal component reported in hospitalized patients (4).

It may be that there are genetic differences in the quantity of phosphatase added to the serum from the intestine. Data are not yet available, however, to prove this point. The present study indicates that under proper circumstances, intestinal phosphatase can be found in the serum of non-secretors and type A subjects. It also suggests that a study of the cellular action of alkaline phosphatase in the intestinal mucosal cell during absorption of fat would be an important area for future work. Pertinent here are histochemical studies performed on the intestinal mucosa of rats during lipid absorption (15). Quantitative determinations of alkaline phosphatase of rat serum during fat absorption show an increase in activity over the fasting (16). Electrophoretically, the serum has a single band which corresponds to the major band in extracts of rat intestinal mucosa (Warnock, unpublished). The histochemical studies have shown an increase in phosphatase in the mucosal cells of the jejunum 4–6 hr following administration of cream via stomach tube (7). This increase is represented histochemically by a diffuse reaction for phosphatase throughout the cytoplasm of the mucosal cell, whereas under fasting conditions activity is restricted to the brush border and Golgi zone. The mechanism whereby this increase occurs and whereby the enzyme enters the serum is yet unknown.

Summary. After administration of a meal, containing 56 g of fat, serum alkaline phosphatase of intestinal origin was demonstrated electrophoretically in 14 subjects lacking it in the fasting state. Other subjects ingesting skim milk or glucose did not develop the intestinal component. A dilution technique indicating the sensitivity of the electrophoretic method is presented and shows that 2–3 mU/ml of phosphatase activity can be detected. Because of this sensitivity, intestinal phosphatase could be demonstrated in types of subjects in whom it had not been found by other authors. The importance of diet in studies of intestinal phosphatase is emphasized.

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Intestinal Absorption of Heparin in the Rat and Gerbil (33422)

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The general impermeability of the gastrointestinal tract to aqueous solutions of heparin has long been recognized (1), and can be satisfactorily explained on the basis of the physical properties of the heparin molecule, namely, its high molecular weight and acidic nature. As a rule, water-soluble compounds of molecular weight greater than 200, penetrate the mucosal cell only with considerable difficulty, and ionized compounds are generally absorbed at a rate much lower than that of neutral molecules (2).

The present work demonstrates that absorption of heparin can occur when the polysaccharide is presented to the intestinal mucosa of rats and gerbils in the form of an oil-in-water (O/W) emulsion. The characteristics and chemical requirements for this phenomenon are herein reported and possible explanations are considered.

Materials and Methods. Materials. Emulsions were prepared with the following oils: corn or olive (Fisher), peanut (Welch, Holme and Clark), mineral, (Lederle) or trioctanoin (Eastman). Monoolein was obtained from Eastman. Water-soluble surfactants used were sodium taurocholate (Wilson), cholic acid or sodium glycocholate (Nutritional Biochemicals Corp.), polysorbitan monolaurate, "Tween-20" (Mann), and a phosphate ester surfactant, "RE-610" (Antara Chemicals, Division of General Aniline and Film). The water-soluble polysaccharides studied were heparin, sodium (Lederle) and mactin B, a heparin-like polysaccharide obtained from the clam, *Cyprina* (Arctica) *islandica* (3). "Ediol," a commercial coconut oil emulsion, was the generous gift of Riker Laboratories.

Preparation of emulsions. Prior to emulsification, heparin, sodium, and a water-soluble surfactant were dissolved in the aqueous phase. Oil-soluble components were dissolved in the oil phase. Emulsification was then carried out with either the Raytheon Sonic Oscillator Model DF-101 or the Branson Sonifier, Model S-125. The oil phase (4.5 ml) and its components were slowly added to the aqueous phase (31.5 ml) in 1-ml increments and emulsification continued until the oil phase was completely dispersed. This required about 5 min in the Raytheon Sonicator and a few seconds in the Branson Sonifier.

Preparation of micellar solutions. Micellar solutions (4) were prepared by dissolving 803 mg of monoolein in 50 ml of a solution containing sodium taurocholate (.02*M*), sodium glycocholate (.02*M*) and 30 mg heparin/ml. A clear solution was obtained upon shaking the mixture at 37° for 1.5 hr.

Administration. Animals employed were CFE male rats (*Carworth Farms*) (150–250 g) or male Mongolian gerbils (50–100 g) from our own colony. All preparations were administered intraduodenally at a dose of 100 mg heparin/kg body weight in volumes of 5.0 or 10.0 ml/kg body weight.

Animals were anesthetized with 60 mg/kg "Diabutal" (*Diamond Laboratories*). The duodenum was exposed through a midline incision and a loop of surgical thread loosely placed around the duodenum about 1 cm distal to the pylorus. The preparation was then introduced via a blunt needle inserted into the duodenum between the pylorus and the loop. Before injection, the needle was advanced until the end of the needle was distal to the loop. The duodenum was then held gently closed at the loop, the prepara-