TABLE II.
Activity of Cells Grown for 1-3 Days in Acetate Medium.

Substrate	Cells harvested after 1 day Q_{O_2}	2 days Q_{O_2}	2.5 days Q_{O_2}	3 days Q_{O_2}
Glucose	33.3	38.6	40.9	15.4
Glycerol	66.5	101.5	116.1	64.7
Pyruvate	88.8	38.2	23.5	13.6
Lactate	31.0	19.8	21.4	13.1
Butyrate	21.8	15.6	9.6	8.6
Acetate	203.3	100.3	74.6	30.4
Glycolate	5.5	13.4		2.5
Glyoxalate	24.0	23.1		13.4
Oxalate	0	6.2		0
Glycine	67.3			
Citrate	6.8	8.9		4.0
α-ketoglutarate	8.0	5.9		14.6
Succinate	2.5	5.0		4.0
Fumarate	11.5	1.0		1.0
Malate	27.8	27.8	30.5	14.6
Mg (dry wt) cells per vessel	4.00	4.04	3.84	3.96

usually shown after 18-24 hours growth(6). With this organism, however, the activity with some substrates was greater after 48-60 hours growth (Table II).

TABLE III.
Lag in Acetate Oxidation by Cells Harvested After 2 Days.

Time, hr	Activity of cells harvested after 2 days Q_{O_2}	2.5 days Q_{O_2}	3 days Q_{O_2}
1st	100.3	74.7	30.4
2nd	120.7	139.8	49.8
3rd		147.0	54.2

The activity on different substrates depends not only on the growth medium and the age of the culture, but also on the duration of the test (Table III).

These results would indicate the need for similar studies with other organisms and raises the question as to what might be found during the logarithmic phase of growth.

Summary. The variations in metabolic activity of the soil organism, *Corynebacterium creatinovorans* (Strain "NC"), grown in media containing different carbon sources is described.

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An Antichylomicronemic Substance Produced by Heparin Injection.* (18042)

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Heparin *in vivo* abolishes alimentary lipemia. This effect is not obtained when heparin is added to blood *in vitro*(1-3). Perfusion of heparinized lipemic blood through different parts of the body results in the disappearance of the lipemia suggesting that no specific organ is involved(2). The effect observed is due to a change in the physical state of the fat since the total plasma lipid level is not altered during short term experiments. Heparin injection has also been found to increase the rate of fat uptake from the intestine(3). In the present work the possibility that a surface active substance is produced by heparin administration has been explored.

The chemical composition of heparin would not suggest surface activity(4), and surface tension measurements on 8 heparin preparations in aqueous solution (1 mg/ml) showed none was present. Preliminary experiments

* Heparin was kindly provided by Dr. D. W. MacCorquodale of the Abbott Research Laboratories and Dr. L. L. Coleman of the Research Division, The Upjohn Co.

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TABLE I.
Change in Plasma Percent Transmission and Surface Tension After Injection of 50 mg of Heparin. Surface tension measured in dynes/cm.

	% transmission (700 μ)			Surface tension (S.T.)				
		Post-heparin			Post-heparin			
Exp. No.	Pre-heparin	30 sec.	5 min.	Pre-heparin	30 sec.	5 min.	% T. increase	S.T. drop
1*	49.2	69.8	57.9	51.6	50.1	50.6	8.7	1.0
2	33.1	37.3	65.7	56.3	54.5	49.6	32.6	6.7
3	54.3	60.7	81.0	54.6	52.1	46.7	26.7	7.9
4	13.3	37.2	66.1	56.1	45.0	44.8	52.8	11.3
5	67.0	86.0	85.0	52.5	51.8	51.4	18.0	1.1
6	68.0	—	86.8	56.9	—	56.6	18.8	0.3
7	70.0	—	85.2	59.2	—	51.6	15.2	7.6
8	46.2	—	80.2	56.7	—	53.1	34.0	3.6
9	64.2	—	85.9	54.4	—	50.8	21.7	3.6
10	76.2	—	87.1	53.9	—	51.8	10.9	2.1
11	76.2	—	83.9	53.7	—	51.7	7.7	2.0
12	75.0	—	86.0	58.2	—	54.1	11.0	4.1

* Only 25 mg heparin inj. in this instance.

in the dog and the human with preservative free heparin solutions demonstrated that the antilipemic effect is due to heparin itself, and not to the preservative (usually phenol). The remaining experiments were performed with heparin solutions as supplied commercially.

The effect of heparin injection on plasma absorption spectrum and surface tension was studied in twelve experiments in healthy human adults. Three to 4 hours after a fatty meal a blood sample was obtained by venipuncture. Heparin was then introduced through the same needle. Additional blood samples were obtained at 30-second and 5-minute intervals after the heparin was injected. In some instances only one post-heparin sample was taken at 5 minutes. All blood samples were citrated and centrifuged at once. Control experiments with defibrinated blood demonstrated that citrate has no adverse effect. In one instance 25 mg of heparin was administered; in the rest a standard dose of 50 mg was used. The optical transmission of the plasma over the range 400 mμ to 700 mμ was determined in all instances. Optical measurements were made in 10 x 75 mm round cuvettes in a Coleman Junior Spectrophotometer. Surface tension measurements were made on undiluted plasma with the duNoüy tensiometer using the special precautions recommended(5).

5. duNoüy, P. L., *Surface Equilibria of Biological and Organic Colloids*, N.Y., 1926.

The results are shown in Table I. The surface tension drop varied from 0.3-11.3 dynes/cm after heparin injection, and the percent transmission rise varied from 7.7-52.8% at 700 mμ. In the instances where cholesterol was followed, no significant change was observed. The detailed results of one experiment are shown in Fig. 1. It is evident that a decrease in surface tension accompanies the heparin-induced clearing of lipemic plasma. This suggests that a surface active agent is formed when heparin is injected. In fact, the presence of this antilipemic substance can be demonstrated *in vitro* by the loss of turbidity occurring when lipemic pre-heparin plasma and cleared post-heparin plasma are mixed. An experiment demonstrating this is shown in Fig. 2. It should be noted that little change in optical density takes place in the plasmas from which the mixture was made. The antilipemic surface active substance, though formed only *in vivo*, clears lipemic plasma both *in vivo* and *in vitro*.

Contrary to previous reports(3) the turbidity did not return to heparin-cleared plasmas left 24 hours at room temperature. Instead continued clearing was noted. The difference between pre- and post-heparin plasmas is more marked if the samples are refrigerated since the low temperature causes the lipemic plasma to become more opaque.

Alcohol extracts of heparin cleared and

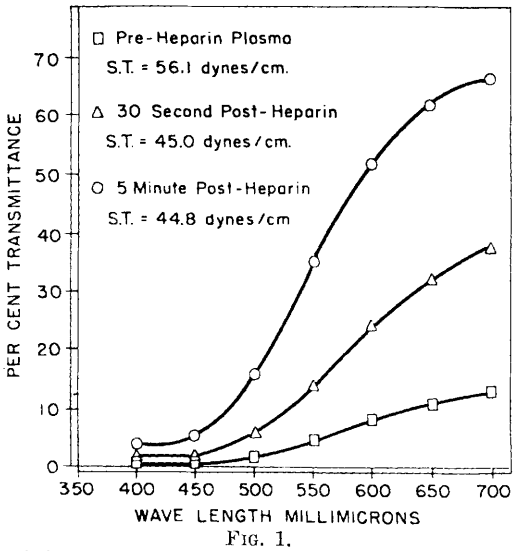

FIG. 1.

Effect of injecting 50 mg of heparin intravenously on plasma absorption spectrum and surface tension (S.T.).

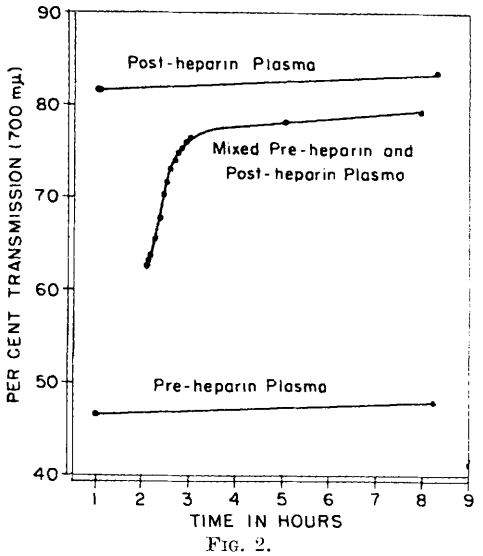

FIG. 2.

Effect of mixing pre-heparin lipemic serum with cleared serum obtained from the same individual 5 minutes after injecting 50 mg of heparin intravenously.

uncleared plasmas showing little difference in surface tension were evaporated to dryness and then shaken up in water. The post-heparin preparations had a surface tension about 10 dynes/cm lower than the corresponding pre-heparin plasma preparations. More striking than the surface tension drop was the soapy nature of the post-heparin preparation demonstrated by the formation of a persistent foam. Since plasma proteins were removed in making these preparations, protein would not seem to be a constituent of the surface active material present.

n-octylamine heparinate, a slightly soluble heparin salt(6), exhibits considerable surface activity in very dilute solutions. While this observation is suggestive, the surface activity of n-octylamine chloride itself makes experimental work with the heparin salt inconclusive. The surface activity of heparin in combination with choline containing lipids was therefore studied.

When heparin was added to a colloidal egg lecithin solution no decrease in turbidity was noted. In agreement with previous findings(7) a solution containing 5.1 mg/ml of fresh egg lecithin was found to have essentially the same surface tension as water. The addition of heparin did not alter the surface tension. When heparin and lecithin were precipitated together from an alcohol solution by acetone a surface active material was obtained which gave a low surface tension in water and formed a soapy solution. The lecithin used in these experiments was freshly prepared and was reprecipitated with acetone four times. However, it is not sufficiently pure to exclude the possibility that other substances may be responsible for this effect. The failure of heparin to form a surface active combination with lecithin when mixed with it in water may be due to the presence of impurities or to the colloidal nature of the lecithin under these circumstances—a factor which may also prevent heparin from forming a complex with the basic phospholipids of lipemic blood *in vitro*. Whether heparin combines only with oriented phospholipids of the cell surfaces lining the vascular system, combines with blood phospholipids only under conditions existing in capillary beds, or is enzymatically combined with phospholipids, cannot be profitably con-

6. Jacques, L. B., *Acta Haematologica*, 1949, v2, 188.

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sidered at this time.

It should be noted that when the aqueous lecithin solution used above was allowed to stand 2 weeks it became highly surface active. Whether this change is correlated with the oxidation of the lecithin or not is not known.

Discussion. Measurement of the surface tension reducing activity of a substance is not a true indication of its emulsifying and detergent properties. This is especially true when measurements are made in the presence of a large amount of fat as is the case here. However, such measurements constitute the only direct approach available. The fact that any surface tension lowering of plasma is observed is surprising since the surface active material might be expected to follow the fat and not accumulate at the air-water interface.

The surface activity ascribed to lecithin is believed to be due to the presence of polysaccharide impurities(7) since solutions of pure lecithin show little surface activity. Commercial lecithin preparations contain large amounts of carbohydrate some of which cannot be removed(8). Lecithin has also been reported to occur in loose combination with carbohydrate in plants(9). MacLean(10) in attempting to fractionate a phospholipid preparation of animal origin discovered a polysaccharide derivative which we know as heparin. The findings reported in this paper are therefore suggested to some extent by the initial work of MacLean, and the association of phospholipids with polysaccharide derivatives such as heparin may be a very general phenomena.

The surface active agent responsible for the clearing of lipemic plasma *in vivo* and *in vitro* may be a heparin-phospholipid complex. The highly acidic heparin molecule may possibly be attached to the extremely basic choline of a phospholipid similar to lecithin, which has a theoretical isoelectric point of 7.5(11). The effects of choline deficiency on fat transport, insulin sensitivity, and liver fats are well known(12) and may be due in part to a failure to produce the surface active substance described here. The possible relationship of the findings reported here to pathological conditions related to chylomicronemia such as atherosclerosis(13) and the lipemia occurring in diabetes and acute pancreatitis deserve special attention.

In view of the deleterious effects of intravenous fat injections due to the size of the fat droplets and the uptake of fat by the reticulo-endothelial system(14), the use of heparin and heparin-phospholipid complexes for therapeutic fat administration is being investigated.

Except in instances where normal blood heparin levels exist, the use of chylomicron counts to follow fat absorption is questioned.

The surface active, chylomicron dissolving effect demonstrated has been tentatively termed achylin activity.

Summary. The injection of heparin (50 mg intravenous) in the human causes a sudden fall in surface tension and a clearing of the lipemic plasma. The effect is believed to be due to the formation of a surface active heparin-phospholipid complex. The activity of the complex may be studied *in vitro* by mixing lipemic plasma with non-turbid plasma obtained after heparin injection. A gradual clearing is observed. The formation of a surface active heparin-egg lecithin complex has been demonstrated. Certain relations of these findings to pathological conditions are discussed.

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