trary to general beliefs concerning the role of bile acids in fat absorption, but many speculative possibilities await study. Further direct experimentation is required to determine (a) whether the absorptive defect consequent to bile exclusion can be completely corrected, (b) the identity of the active constituent(s) in bile (c) the effect, if any, of the manner of entry of active material into the intestine, (d) the role, if any, of species differences, and (e) the mechanism by which fat absorption is facilitated.

Summary. 1. Three-gram doses of certain bile preparations (including desoxycholic acid) proved completely ineffective in reducing the steatorrhea of bile fistula dogs.

2. Ninety-cc doses of fresh ox bile reduced the steatorrhea by 50%, but equivalent doses (6 g) of desiccated ox bile were only half as effective.

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Increased Hemolytic Potency of Mouse Mammary Carcinoma Extracts Following Incubation with Tumor Cells.*

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It was recently observed in this laboratory that filtered or centrifugated extracts prepared from spontaneous mouse mammary carcinomas possess the ability to hemolyze mouse erythrocytes *in vitro*.[1]

In course of subsequent experiments it was found that tumor extracts that had been separated from the tumor cells by centrifugation, and have been left, in absence of cells, in test tubes, at room temperature, lost practically all hemolytic potency after 5 hours; the decrease in the hemolytic potency of the tumor extracts was even more rapid at 37°C.[2] This rather striking lack of resistance of the hemolytic factor contained in the tumor extracts, but separated from the tumor cells, to exposure for a few hours to either room, or incubator temperature, was further investigated. One of the first questions requiring clarification was whether the tumor extracts would also lose their hemolytic potency if incubated, at 37°C, in presence of tumor cells.

Accordingly, a series of experiments was performed in which tumor cell suspensions of 20% concentration were freshly prepared from spontaneous mouse (C3H) mammary carcinomas.[2] Fresh samples of such suspensions were tested for their hemolytic activity.[1] The suspensions were immediately divided into 2 groups of tubes: one was left unchanged as a control, and the other was centrifugated twice at 3,000 t.p.m. for 10 minutes each, to separate the extracts from the tumor cells; following centrifugation, the final supernatant fluid was used. Both, the tumor cell suspensions, and the cell-free extracts, were then placed in an incubator, at 37°C, for 5, and 24 hours. After incubation, samples removed from both groups of tubes were tested for their hemolytic activity by serial titration. Eight different mouse mammary carcinomas were used for the preparation of extracts, and 8 successive series of experiments were performed under apparently identical conditions. The results were as follows:

All 8 fresh tumor extracts were found to possess the usual hemolytic potency[1] when tested before exposure to the incubator temperature. After exposure for 5, or 24 hours, to 37°C, the centrifugated tumor extracts incubated without cells, were found to have lost their hemolytic potency, when tested in

* Published with the permission of the Chief Medical Director, Department of Medicine and Surgery, Veterans Administration, who assumes no responsibility for the opinions expressed or conclusions drawn by the author.

1 Gross, L., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 292.

2 Gross, L., *J. Immunol.*, 1948, in press.

the usual[1] manner (0.5 cc of an extract of 20% concentration). On the other hand, the same tumor extracts incubated in presence of tumor cells, at 37°C, for either 5 or 24 hours, had not only their hemolytic potency preserved, but either moderately or substantially increased, as compared with the initial titration of fresh extracts. Thus, one of the fresh extracts was found to hemolyze mouse erythrocytes only when tested (0.5 cc) in 20% concentration, *i.e.*, at 1:5. Following incubation in presence of tumor cells at 37°C for 5 hours, the same extract (0.5 cc) hemolyzed readily mouse erythrocytes in dilutions up to 1:40. After an additional incubation with tumor cells at 37°C to a total of 24 hours, this extract hemolyzed mouse erythrocytes in dilutions up to 1:160.

In some instances the increase in hemolytic potency appeared to be slightly more pronounced after 5 than after 24 hours of incubation with tumor cells. In one experiment an extract which had been found to hemolyze mouse erythrocytes, when tested prior to incubation, only at either 1:5, or 1:10, had after 5 hours of incubation at 37°C with tumor cells its hemolytic potency increased to such an extent that serial dilutions up to 1:160 gave positive readings. After an additional incubation with tumor cells to a total of 24 hours, however, the hemolytic potency of the same tumor extract was slightly decreased, giving positive readings in dilutions not exceeding 1:40; thus, it was still higher than that of the initial titration of the fresh tumor extract, but less pronounced than that tested after 5 hours of incubation.

Control Experiments with Normal Cells. It was previously observed[1,2] that fresh extracts prepared from normal mouse organs, such as liver, spleen, kidneys, muscle, or lungs, had no hemolytic potency on mouse erythrocytes *in vitro* in practically all instances tested,† except for extracts made from active mammary glands removed from nursing or pregnant female mice of either a high-tumor, or a low-tumor line.[2]

In the present study additional experiments were performed in which normal mouse cell suspensions were incubated at 37°C for either 5 or 24 hours, and then tested for hemolytic potency. Accordingly, normal organs, such as liver, spleen, kidneys, muscle, and lungs, were removed from a total of 12 healthy, adult, virgin female mice of the An (C3H)[3] subline, and either individual or pooled cell suspensions of 20% concentration were prepared from each mouse. Fresh samples of the extracts were tested for hemolytic activity.[1] The cell suspensions were incubated at 37°C for either 5 or 24 hours; after this lapse of time, the suspensions were centrifugated, and the supernatant extracts were tested for hemolytic potency in the usual manner.[1] The results were as follows:

Fresh samples of the normal cell extracts had no hemolytic potency.† This result was essentially consistent with previous observations.[1,2]

With the extracts obtained from normal cell suspensions that had been *incubated for 5 hours,* the following readings were obtained: Of 8 individual liver extracts tested (0.5 cc), 1 was positive in 1:10 dilution. Of 8 pooled (kidney, spleen, muscle, lungs) extracts tested (0.5 cc), 3 were positive in 1:10 dilution. All other extracts, including 4 groups of cell suspensions made from individual organs (lungs, spleen, kidneys, muscle) of 4 females, were negative.

A few tests were made with extracts obtained from cell suspensions that had been *incubated for 24 hours.* Two of 4 liver extracts, and 2 of 4 pooled extracts were positive (0.5 cc) at 1:10. One of 3 lung extracts tested was positive at 1:5, and 1 of 3 muscle extracts at 1:5, and 1:10. All other extracts, including 3 spleen, and 3 kidney extracts, were negative.

Summary. If separated from the tumor cells by centrifugation, the mouse mammary

† This was true under routine conditions of the test, *i.e.*, incubation of the extracts with mouse erythrocytes at 37°C for 2½ to 3 hours.[1] It was found, however, that normal mouse organs may occasionally show a "delayed" hemolytic potency; thus, when extracts prepared from normal mouse cells were mixed with mouse erythrocytes, and incubated first at 37°C for 5 hours and then at 7°C (refrigerator) for additional 15 hours, a slight hemolysis resulted in some instances.

[3] Gross, L., *J. Immunol.*, 1947, **55**, 297.

carcinoma extracts lose their hemolytic potency, when incubated at 37°C, after 5 hours. On the other hand, the hemolytic potency of the tumor extracts is either moderately, or substantially increased following incubation in presence of tumor cells, at 37°C, for either 5 or 24 hours.

Fresh extracts prepared from normal mouse cells have in most instances no hemolytic potency, except for those made from active mouse mammary gland.[2] If the normal mouse cell suspensions (liver, lungs, muscle, or pooled organs), however, are incubated at 37°C for 5, or better for 24 hours, some of them may show a slight hemolytic potency, though to a lesser degree than that observed in the case of pre-incubated tumor cell suspensions.

16300

Reconstitution of the Dermal Barrier to Fluid Diffusion Following Administration of Hyaluronidase.*

OSCAR HECHTER. (Introduced by David Rapport.)

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It is now generally accepted that hyaluronidase owes its spreading activity to the action of the enzyme upon a viscous hyaluronic acid gel in connective tissues[1,2] which is one of the components of the tissue barrier to interstitial fluid diffusion.[3] The effect of hyaluronidase in facilitating the spreading in skin remains evident in the locally treated area for as long as 24 hours after administration of the enzyme, and thereafter is absent.[4] This demonstrates that after a certain latent period, the organism replaces the component of the skin barrier removed by the hyaluronidase.

It appeared likely that a quantitative study of the rate at which the dermal barrier to spreading of fluids was restored, after administration of hyaluronidase, might provide information concerning the formation of new dermal hyaluronic acid *in vivo*. Accordingly, such a study was undertaken in adult humans.

The hyaluronidase used was a purified bovine testis preparation (Schering) which assayed 20 turbidity-reducing units (TRU) per mg as evaluated by the method of Kass and Seastone.[5] The enzyme was used directly after dissolution in 0.85% sodium chloride. Three men and 3 women received intradermal injections of 0.20 cc of enzyme solution containing 20, 2, 0.2, 0.02, 0.002, and 0.0 TRU per cc into separate areas of the 2 arms. After the injection of the enzyme solutions, the local areas were marked with tincture of merthiolate. The time of wheal disappearance following intradermal injection served as a measure of hyaluronidase spreading activity. The local area was examined continuously for 10 minutes after the injections, then at 5-minute intervals until the wheal has disappeared, *i.e.*, until the injected local area appeared completely flat as viewed from a 90° angle. At 24, and then again at 48, hours after the initial injections of enzyme 0.20 cc of an 0.85% solution of NaCl was injected into each previously treated area, and the time of wheal disappearance determined.

Table I illustrates the interrelationships between the time required for wheal disappearance and the concentration of enzyme injected. On the assumption that the time of wheal disappearance is a measure of the barrier efficiency of the dermal hyaluronic acid gel associated with the tissue barrier to

* Aided by a grant from the G. D. Searle & Co.

1 Duran-Reynals, F., *Bact. Rev.*, 1942, **6**, 197.

2 Meyer, K., *Physiol. Rev.*, 1947, **27**, 335.

3 Hechter, O., *Fed. Proc.*, 1947, **6**, 126.

4 McClean, D., *J. Path. and Bact.*, 1930, **33**, 1045.

5 Kass, E. H., and Seastone, C. V., *J. Exp. Med.*, 1944, **79**, 319.