red cell ACh as a mechanism by which the cells accumulate K is of doubtful importance.

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Chylomicron and Clearing Reaction: Effect of Heat and of Refrigeration.* (21013)

ROY L. SWANK† AND ESTHER S. ROTH.

From the Department of Neurology and Neurosurgery, McGill University and the Montreal Neurological Institute.

The *in vitro* reaction by which turbid fat suspensions may be cleared following the addition of plasma obtained from animals after the injection of heparin has been studied extensively by Anderson and Fawcett(1), Anfinsen *et al.*(2), and French *et al.*(3). The present study is concerned with the fat particles (chylomicra) themselves and with factors which alter their susceptibility to clearing.

Method. Samples of lipemic plasma were obtained from dogs approximately 3 hours after fat meals of 4-8 g/kg. Cream fat was fed except where indicated. The plasma samples were centrifuged (at 4°C for 30 minutes at 20,000 × G.) in a Servall angle centrifuge and the subnatant clear plasma removed. This was replaced by 0.85% NaCl, and the suspension was recentrifuged. This operation was repeated 3 times. The resulting saline suspension of washed chylomicra was used at once or stored at 4°C or −18°C and used within a few days. Chyle was obtained by cannulation of the thoracic duct of dogs 1-2 hours after feedings of 8 g of cream fat per kg, and washed suspensions of chylomicra from the chyle were prepared as described for the plasma. All clearing tests were performed as follows: 1 ml of chylomicron suspension (or lipemic plasma or diluted chyle) plus 4 ml 0.85% NaCl were warmed at 37°C for 10 minutes in a 19 mm Coleman cuvette. One ml of warm post-heparin plasma was then added, the time noted and the turbidity of the suspension determined immediately and at 10-minute intervals for one hour or longer. All determinations of optical density were made in a Coleman spectrophotometer at 650 mμ wave length. The tubes were incubated at 37°C in a water bath during the test. The samples of post-heparin plasma were obtained

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† Now located at the University of Oregon Medical School, Portland.

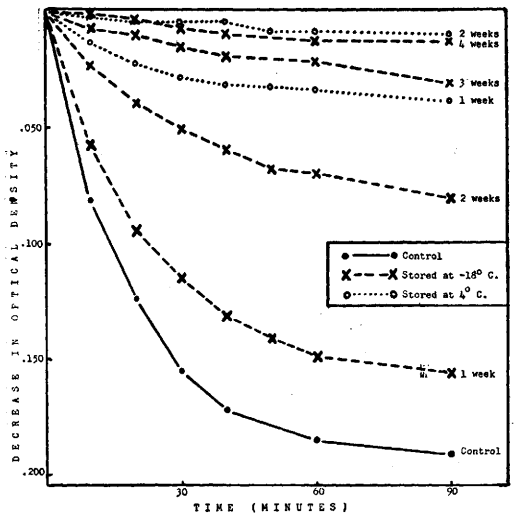

FIG. 1. Clearing factor tests using saline-washed chylomicra which had been stored at –18°C or 4°C for the periods of time indicated.

from dogs 10 minutes after the injection of 25-50 units of heparin (Connaught Laboratories) per kg body weight. These were stored at –18°C until used. We found no decrease in potency of these samples after storage for one month at this temperature. In all cases blood samples were drawn into 1/10 volume 3.8% sodium citrate solution. Chyle was also collected in the same anticoagulant. For the stability experiments 1.0 ml amounts of the chylomicron suspension to be tested were pipetted into tubes and subjected to different temperatures. The entire contents of these tubes were then used for the clearing test. For any one experiment aliquots of the same post-heparin plasma sample were used as the source of "clearing factor."

Results. Effects of refrigerator storage and of freezing. The susceptibility of washed chylomicra or of whole lipemic plasma to clearing by post-heparin plasma decreased both in rate and extent during storage at 4°C or –18°C. This change was marked at the end of one week and increased progressively with the time of storage (Fig. 1). Repeated (3 times) rapid freezing (to –18°C) and thawing (to room temperature) of washed chylomicra during a period of 5 hours had little effect on the susceptibility of the chylomicra to clearing.

Effect of heat. When washed chylomicra were heated to various temperatures no sharp inactivation temperature was observed; a gradual reduction in the susceptibility of different aliquots to clearing occurred as the temperature increased (Fig. 2). A gradual decrease in the susceptibility to clearing also occurred as the period of heating was increased (Fig. 3). Heating at 37°C for 15 minutes always caused a slight decrease in the susceptibility to clearing; a complete loss of the ability to be cleared was present after washed chylomicra had been heated for 3 hours at 100°C, or for 24 hours at 37°C.

Nature of fat in the chylomicra. In one experiment each of 3 dogs (siblings) was fed a different fat: cod liver oil in skim milk (4 g fat/kg body weight), glyceryl trioleate in skim milk (4 g fat/kg body weight) and 35% cream (6 g fat/kg body weight). The chylomicra obtained from each dog were washed 3 times in saline as usual. The samples were adjusted to exactly the same optical density, and aliquots of each were heated at 65°C for 30 minutes. Fig. 4 shows that the chylomicra from the dog fed glyceryl trioleate cleared more rapidly than the chylomicra from the cream-fed dog. The susceptibility to clearing of both suspensions was lessened to about the same degree by heating. Chylomicra from the

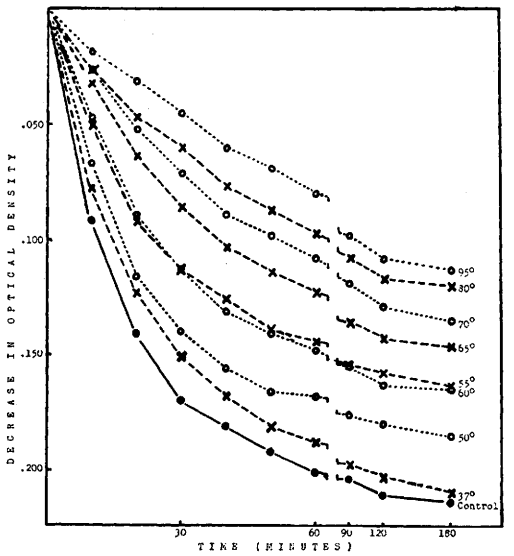

FIG. 2. Clearing factor tests (at 37°C) using saline-washed chylomicra which had previously been heated for 15 min. at the temperatures indicated.

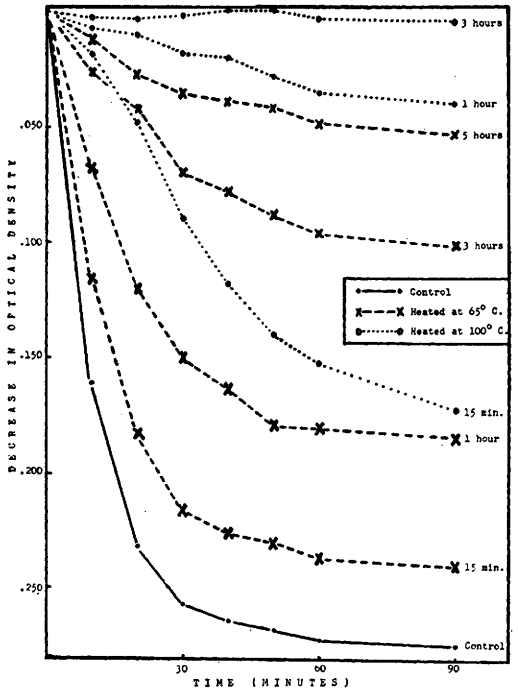

FIG. 3. Clearing factor tests (at 37°C) using saline-weshed chylomicra which had previously been heated at 65°C or 100°C for the periods of time indicated.

cold liver oil-fed dog cleared much less rapidly than did the chylomicra from the other 2 dogs, and after heating to 65°C for 30 minutes these chylomicra lost almost completely their ability to be cleared. Microscopic examination (dark field) of the unheated preparations showed that the "cod liver oil chylomicra" were much larger than the others. It is of interest that *in vivo* cod liver oil lipemias clear less rapidly following the injection of heparin than do lipemias following the ingestion of cream or glyceryl trioleate(4).

Experiments with added plasma. Many attempts were made to determine if the chylomicra which had suffered some loss of their susceptibility to clearing during heating could regain this property in the presence of fresh plasma. Various conditions such as the addition of different amounts of plasma with or without incubation for different periods of time, and centrifuging, washing and re-isolation of the chylomicra after the addition of plasma were tested. The results varied. In about half the experiments the addition of normal plasma to suspensions of chylomicra damaged by heating to 37°C or 65°C was attended by a partial recovery of the rate of clearing of the chylomicra. In no instance was the recovery complete. In a number of experiments 1.0 ml of normal plasma and saline in different proportions was added to 1.0 ml of washed, saline-suspended chylomicra. This was heated and then centrifuged to remove precipitated plasma proteins. The supernatant was tested for its clearing susceptibility. In all experiments the addition of 0.1 to 0.5 ml of plasma reduced the expected loss of susceptibility to clearing when heated to both 65°C and 37°C. The addition of 0.01 ml and 0.02 ml of plasma usually did not give this protection.

Experiments with chyle. Freshly obtained chyle, diluted approximately 1:100 with saline lost only a little of its susceptibility to clearing during heating at 50°C or 65°C for 15 minutes. Also when stored for as long as one month in the refrigerator or at −18°C in its

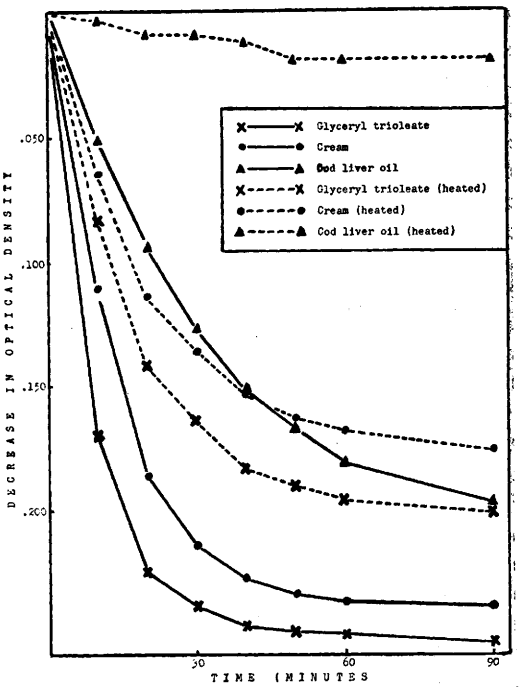

FIG. 4. Clearing factor tests using saline-washed chylomicra obtained from dogs which had been fed 3 different fats (as indicated). Broken lines show the same chylomicra tested after heating 30 min. at 65°C. All optical densities were the same at the beginning of the clearing test.

undiluted state, chyle lost none of its susceptibility to clearing. Saline-washed chylomicra from chyle, on the other hand, were significantly less susceptible to clearing after storage for one month at −18°C, although they suffered less in this respect than did saline-washed chylomicra from plasma. It was found necessary to warm the frozen chyle 2-3 hours at 37°C before testing, otherwise it continued to equilibrate during the testing and erroneously gave the appearance that its clearing susceptibility improved after heating at 50°C or 65°C. After the 3 hours prewarming, however, it was found that the previously frozen chyle showed the same degree of very slight "damage" after 15 minutes heating at 50°C or 65°C as the fresh chyle.

Discussion. Our results showed that the susceptibility of the chylomicra to clearing by post-heparin plasma may be reduced by storage at 4°C or −18°C or by heating. Whether this is due to changes in the fat of the chylomicron or in its protein envelop, or to changes in a lipoprotein complex cannot be concluded from our studies. If the changes are due to denaturation of a protein film on the chylomicron the question would still arise as to whether this film is essential in the sense that it is an essential factor for maximal clearing, or whether the denatured protein coating interferes with the clearing reaction by making the fat unavailable. It is of interest that loss of susceptibility to clearing brought about by heating the chylomicra was not completely reversed by the addition of normal plasma, but the presence of certain amounts of plasma during heating lessened this change.

The possibility must also be considered that a specific protein or lipoprotein complex is involved. This is suggested by the fact that the heating of chyle (diluted 1:100 in saline) caused much less change in the susceptibility to clearing of the chylomicra than the same heat applied to washed chylomicra from lipemic plasma. This might suggest that the protein moiety of the chylomicron is fundamentally important to the clearing reaction, or that chyle itself contains a specific substance necessary to maximal clearing that is either absent or present in relatively low concentration in plasma.

Conclusion. Heat or storage at −18°C is attended by a significant reduction of the susceptibility to clearing of saline washed chylomicra from plasma. Thoracic duct chyle is affected much less by the same treatments.

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Plasma Volume Changes in Egg-White Injected Rats. Effect of Cortisone.* (21014)

FLORENCE WILLIAMS. (Introduced by G. Ungar.)

From Institution for Tuberculosis Research and Department of Physiology, University of Illinois College of Medicine, Chicago.

Selye described an "anaphylactoid" condition produced by intraperitoneal injection of egg-white to rats(1) characterized by the development of edema. Further studies(2,3) have shown that adrenalectomized rats reacted to egg-white with a more severe condition, usually fatal. It was also observed that adrenal cortical extract(4) and cortisone (5) had a protective effect on the systemic actions of egg-white. The present report deals with plasma volume changes in egg-white-treated rats and attempts to explain the mechanism of the protective action of corti-

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