the present study are in accord with their observation that infection with an A_2 strain stimulates antibody production against A and A_1 strains.

Summary. In volunteers the sequential appearance of the immunoglobulin classes of neutralizing antibody formed as a result of infection with A_2/Bethesda/10/63 influenza was studied. The antibody appeared in the IgG class and no significant antibody in the IgM or IgA classes was found. It was particularly notable that no IgM antibody could be detected 7 days after inoculation. The pattern of antibody against A_2/Japan/305/57 was nearly identical to that formed against the inoculum strain. Antibody rises were also noted against A/PR8 and A_1/FM1 influenza viruses.

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Lipid Mobilizer Hormone in Triton Hyperlipemia.* (32082)

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A lipid-mobilizing hormone (LM) was discovered in the plasma of man and other species by Seifter and Baeder(1) in 1954. These workers later isolated LM from the posterior pituitary gland of hogs(2) and demonstrated that the plasma content of LM is greatly increased by administration of cortisone and by subjecting animals to cold or to the nephrotic syndrome(1,3). Lipovetskii reported confirmatory findings for the presence of LM in human plasma(4) as have Kadas and Nagy(5). The latter authors also demonstrated that LM was derived from the posterior pituitary in further confirmation of the reports of Seifter and Baeder.

LM exerts two major effects: (1) it mobilizes triglycerides from the omental and mesenteric depots, and (2) it inhibits the delactescent action of heparin-clearing factor *in vitro*. Extensive studies have shown LM

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to be involved in the elevation of blood lipids which occurs consequent to surgical stress(6), nephrosis(7), pregnancy(8), and hereditary hyperlipemias(7). In addition, the hyperlipemias induced by protamine(9), by diisopropylfluorophosphate(9) and by cobaltous chloride(10) appear to be mediated by the LM mechanism.

The present investigation was undertaken to determine whether LM also participates in the hyperlipemia which follows administration of Triton WR-1339, a non-ionic surface-active agent(11).

Materials and methods. Triton WR-1339 was administered to rabbits intramuscularly in a dosage of 300 mg per kilogram per day. One group of 6 animals received daily injections for 10 days, while 2 groups of 8 rabbits each were given injections only on two successive days. Baseline and periodic blood specimens were drawn from each animal for lipid determinations, electrophoretic estimation of lipoproteins, and for indirect assessment of LM content by inhibition of heparin-activated clearing factor. Details of the tests employed have been given elsewhere(10).

Results. Elevation of the serum lipid components occurred in all rabbits given Triton WR-1339.† In the group which received 10 injections of Triton, the total serum lipids increased from a baseline mean value of 113 mg% at 2 days, 2656 mg% at 7 days, and 3052 mg% at 2 weeks. All lipid fractions measured participated in the increase (Fig. 1). Plasma β-lipoprotein greatly increased after Triton administration and by the end of the first week comprised 99% of the plasma lipoproteins as determined from paper electrophorograms.

Inhibition of heparin-clearing factor activity was detected in tests with serum specimens taken 2 days after the beginning of Triton administration. There was generally a sustained inhibitory effect but with a suggestive lesser degree at 3 weeks. Fig. 2 illustrates the clearing factor inhibition findings in the tests on serum samples from one animal in this group. Control studies with Triton WR-1339 added directly to the clearing-factor test system revealed no inhibition in concentrations up to 25 mg%.

Rabbits receiving Triton injections for only 2 days had comparable but less marked elevation in the serum lipids. Composite results for one group of 8 rabbits are shown in Fig. 3. An increase in all lipid components was detected after the 7th post-injection hour, but with greater increments after 24 hours. Inhibition of clearing-factor activity was pronounced at 24 hours (Fig. 4) and in some instances this effect appeared to precede the plasma lipid changes.

Serum lipoprotein values remained essentially unchanged until the 7th hour post-injection when there followed a progressive rise to practically 100% β-lipoprotein at 24 and 48 hours.

Discussion. The hyperlipemic changes which were induced by administration of Triton in these experiments with rabbits are in agreement with the findings of others(11). Similar results have been described in mice (12), rats(13), guinea pigs(11), and dogs (14). It has been postulated that Triton causes physical or chemical changes in the plasma lipids, leading in turn to their accumulation in the blood. Scannu and Page (14) performed studies which demonstrated that the removal of labeled triglycerides from the circulation was markedly delayed in Triton-treated dogs. Such evidence was not obtained for labeled cholesterol and phospholipids. Increased hepatic production was believed to be the likely explanation for the hypercholesterolemia and hyperphospholipidemia of dogs given Triton.

From the present studies, it is evident that the lipid changes which follow Triton administration are accompanied by the appearance in the circulation of an inhibitor of heparin-clearing factor. As noted previously, *in vivo* release of clearing factor inhibitor has been detected in a variety of stressful states and the active inhibitory substance was shown to be LM (lipid mobilizer). While clearing-factor inhibition is an important property of LM, its major effect is mobilization of triglycerides from the omental and mesenteric depots to the portal circulation. Whether or

† Triton WR-1339 was kindly provided for these studies by Winthrop Laboratories, New York.

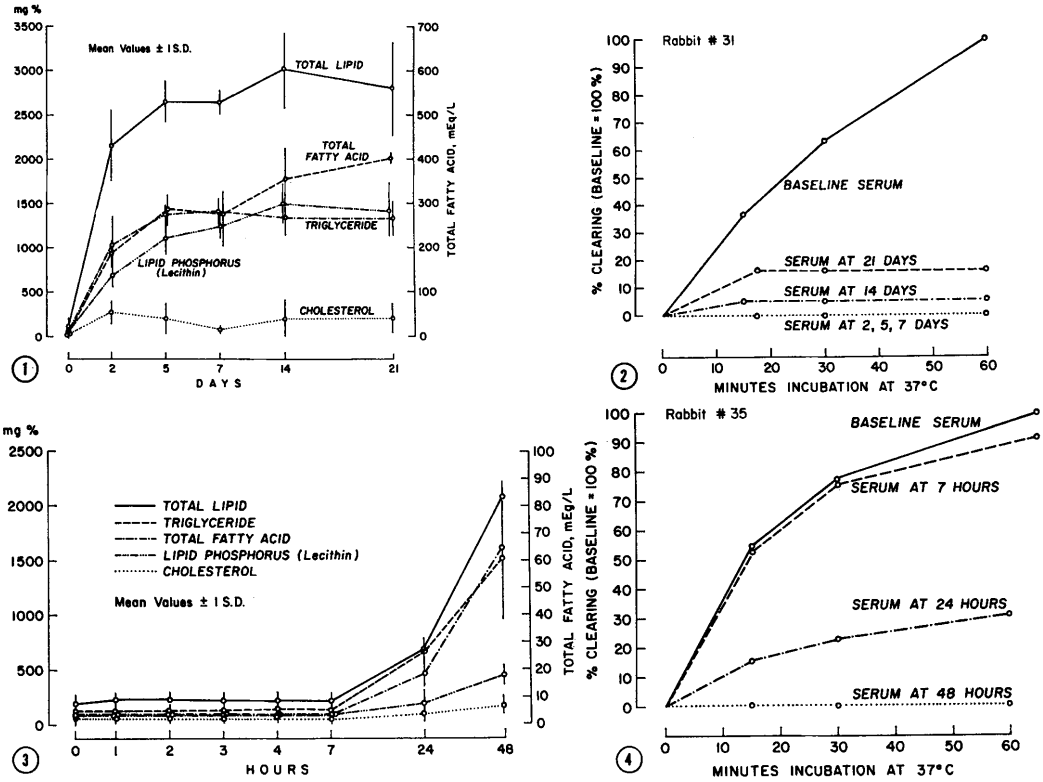

FIG. 1. Composite serum lipid findings in rabbits given 300 mg. Triton WR-1339 per kilogram per day for 10 days. First injection after zero baseline specimen drawn.

FIG. 2. Inhibition of heparin-clearing factor activity by successive serum specimens from animal receiving injections of Triton WR-1339 for 10 days.

FIG. 3. Composite serum lipid findings in rabbits given 300 mg. Triton WR-1339 per kilogram per day for 2 days. First injection after zero baseline specimen drawn.

FIG. 4. Inhibition of heparin-clearing factor activity by successive serum specimens from animal receiving injections of Triton WR-1339 for 2 days.

not hyperlipemia appears in the post-hepatic circulation depends on the ability of the liver to cope with the lipid load presented to it. Certain factors, such as depletion of liver glycogen and traces of hepatotoxic agents, influence the metabolism of this lipid load so that there follows post-hepatic hyperlipemia with elevation in cholesterol and phospholipids as well as fatty acids(15). In these rabbits, therefore, it appears that Triton exerted metabolic effects on liver function which in turn contributed to the lipid findings in the general circulation.

Thus, from the observations in this and related studies, it may be postulated that Triton toxicity at the tissue level creates the stressful stimulus which leads to the release of LM. At the same time, Triton affects the manner in which the liver deals with the triglyceride load it receives *via* the portal circulation.

The possibility that clearing-factor inhibition *per se* causes hyperlipemia is not supported by evidence from previous studies. In this connection, it should be noted that the lipid mobilizing action of LM can be prevented by a partially depolymerized hyaluronic acid (PDHA). In PDHA-treated human subjects, as well as in laboratory animals, there has resulted no lipemia when circulating LM has been shown to be present in strong concentrations and highly inhibitory to clearing-factor action(16).

Summary. Tests were performed on baseline and periodic blood samples obtained from rabbits treated with intramuscular injections of Triton WR-1339. The ensuing hyperlipemia was characterized by marked ele-

vation in total fatty acids, triglycerides, cholesterol, lipid phosphorus, and total lipids. Paper electrophoresis revealed a concomitant increase in β-lipoproteins. Lipid mobilizer (LM) also increased strikingly, as was determined indirectly by testing plasma aliquots for inhibition of heparin activated clearing lipase. On the basis of these and related studies, is is suggested that Triton hyperlipemia is produced, at least in part, through sustained release of increased amounts of LM which mobilize triglycerides to the portal circulation. Furthermore, Triton modifies metabolic activity of the liver so that post-hepatic hyperphospholipidemia and hypercholesteremia ensue.

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A Simple and Inexpensive Device for Restraining Rodents.* (32083)

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In many experimental procedures involving rats or mice, regularly timed blood specimens must be obtained. The use of the tail veins as a source of this blood necessitates incapacitating the animal in either elaborate restraining devices or wrapping them in toweling. Both techniques are often traumatic to both animal and experimenter. A simple device is described which alleviates this problem.

An ordinary low-density narrow-mouth polyethylene bottle is cut with a razor blade as in Fig. 1. Enough of the bottle is left intact to allow the rat to brace his hind legs. Also an injection port may be cut in the side if necessary. The animal needs very little inducement to enter the bottle and frequently will walk in to it of its own accord if the bottle is merely placed before it. Once the rat is in with its tail still out, a wide piece of adhesive tape is used to close the entrance. The size of the bottle should be commensurate with the size of the rat; an 8 oz bottle will conveniently hold a 200 g rat.

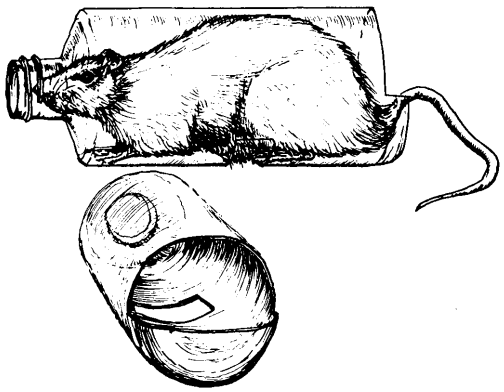

FIG. 1.

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