half-lives, we endeavored to quantitate the time-action curves of these 2 drugs using tachycardia and salivary-suppression in mice. However, difficulties of obtaining precise time-action curves led us to abandon these experiments and to pursue our studies of these 2 drugs using different species. Current approaches also include anticholinergic Q_{10}'s in poikilotherms and simulation of experimental curves on an electronic analog computer.

Summary. The mydriatic half-life in mice of SKF 5515, a new tertiary ammonium anticholinergic drug, was independent of dose and route within the limits of experimental error. The apparent half-life of the methobromide quaternary derivative was dependent upon log dose and route, possibly as a result of decreased rate of penetration of membrane barriers by the charged ion.

I am grateful to Gordon Black for technical assistance, and to Dr. Joseph Swintosky, who initially stimulated my interest in pharmacokinetics. The 2 compounds reported above were synthesized by Dr. Charles Zirkle.

1. Clark, A. J., *Handbuch exp. Pharmakol.*, 1937, v4.

2. Teorell, T., *Arch. int. Pharmacodyn.*, 1937, v57, 205 and 226.

3. Druckrey, H., Küpfmüller, K., *Dosis und Wirkung*, Aulendorf/Württ, 1949.

4. Dost, F. H., *Der Blutspiegel. Kinetik der Konzentrationsabläufe in der Kreislaufflüssigkeit. Leipzig*, 1953.

5. Swintosky, J. V., *J. Am. Pharm. Assn., Sci. Ed.*, 1956, v45, 395.

6. Wilfon, J. G., Macko, E., *Fed. Proc.*, 1959, v18, 458.

7. Dost, F. H., *Klin. Wschr.*, 1958, v36, 655.

8. Herxheimer, A., *Brit. J. Pharmacol. Chemother.*, 1958, v13, 184.

9. Ariëns, E. J., van Rossum, J. M., Simonis, A. M., *Arzneim.-Forsch.*, 1956, v6, 282, 611, 737.

10. Hill, A. V., *J. Physiol.*, 1909, v39, 361.

11. Gehlen, W., *Arch. exp. Path. Pharmakol.*, 1933, v171, 541.

Received March 9, 1960. P.S.E.B.M., 1960, v104.

Comparison of Heparin Induced Lipid Clearing Activity in Human Thoracic Duct Lymph and Plasma.* (25750)

ALLAN E. DUMONT (Introduced by J. H. Mulholland)

Dept. of Surgery, N. Y. University College of Medicine and Third (N. Y. Univ.) Surgical Division, Bellevue Hospital

Plasma from humans and other species after intravenous heparin administration (post-heparin plasma) when incubated with lipemic serum or milky lymph, produces a decrease in optical density, or "clearing" of fat(1). Rate of decrease in optical density (clearing activity) of such lipemic substrates has been used as index of lipoprotein lipase activity in post-heparin plasma(1,2,3). We tested for clearing activity in human thoracic duct lymph to compare it with that in plasma of the same individual.

Methods. Five patients were studied. Two had disseminated cancer, 2 had Laennec's cirrhosis, and the 5th had dyspeptic symptoms following cholecystectomy 3 years previously. The thoracic duct was cannulated in the neck by technic of Linder and Bloomstrand(4). The observations were made on 1st or 2nd post-operative day. Samples of plasma were collected before and 5 and 10 minutes after administration of 10 mg of heparin to 2 patients and 50 mg to the other 3. Consecutive 10 minute samples of lymph were collected continuously before, during and after heparin administration. Furthermore 3 received intravenous heparin 1 day prior to operation, and these post-heparin plasma samples were assayed for clearing activity. Specimens were collected in chilled test tubes containing oxalate and were kept in ice bucket until centrifuged at 30,000 rpm for 15 minutes in refrig-

* This work supported by grants from U.S.P.H.S. Pancreas Study and N. Y. City Dept. of Health. Technical assistance of Amalia Martelli is gratefully appreciated.

erated centrifuge and then stored at −4°C. Clearing activity was determined by turbidiometric method as modified by Robinson and French(5,6). A stock supply of human milky lymph was used as substrate; this was stored frozen following its collection from a patient 3 months previously, and diluted 1:100 with physiological NaCl solution to give optical density readings of less than .7 in Bausch and Lomb colorimeter (Spectronic 20). Plasma and lymph samples were thawed at 4°C and incubated at 37°C in water bath 5 minutes. Six cc of substrate (also incubated at 37°C for 5 minutes) were then added to 1 cc of sample tested and optical density read immediately at wavelength of 640 μ in the colorimeter. Tubes containing the mixture were then returned to water bath and readings made at 15 minute intervals for 1 hour. One tube of substrate without plasma was used as control and a tube of saline as blank. Clearing activity was expressed in units calculated as amount of decrease in optical density minute.

Results. In all 5 patients, only plasma samples collected after heparin administration promoted clearing activity. There was no clearing activity in thoracic duct lymph *in vitro* or *in vivo*.

In 3 patients whose plasma response was determined both before and during duct cannulation, the same degree of clearing activity was demonstrated at both times. Samples of lymph collected 20 and 30 minutes after heparin administration failed to demonstrate clearing activity. Results in 1 patient, which are characteristic of the others, are shown in Fig. 1.

Discussion. Inability to demonstrate clearing activity in human thoracic duct lymph after administration of heparin in quantities sufficient to cause appearance of this activity in plasma is not in accord with results reported by Young and Freeman in rats and rabbits(7). This discrepancy is perhaps due to differences in amount of heparin used, since on a weight basis the animals received at least 10 times the dose employed in these patients.

Robinson and French(8) reported that ligation of thoracic duct in rats increased amount of clearing activity in post-heparin

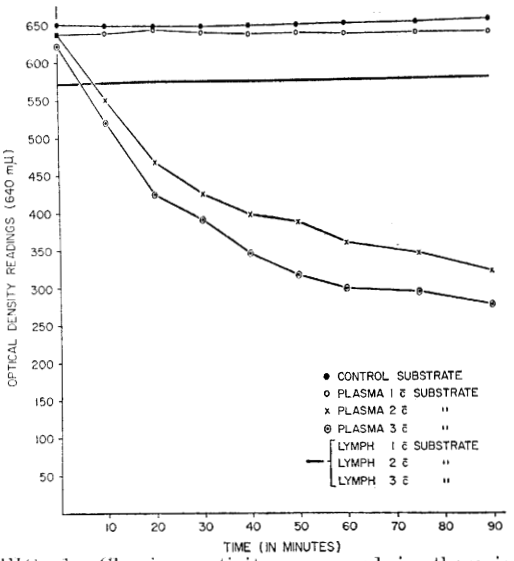

FIG. 1. Clearing activity compared in thoracic duct lymph and in plasma after heparin administration. Results in this patient are representative of results in the other 4.

plasma. They suggested that this increase represented decreased utilization of clearing factor *in vivo* when fatty chyle is prevented from entering the blood. Although thoracic duct was not ligated in our experiments, complete diversion of thoracic duct lymph did not change post-heparin plasma clearing in 3 patients tested before and during cannulation. It seems unlikely therefore that the explanation offered by Robinson and French is correct.

Heightened plasma clearing response to heparin has been reported in patients with chronic liver disease(9). Two patients with cirrhosis of the liver had no different response in either plasma or lymph from the other 4 patients.

Rate of decrease in optical density of a substrate consisting of milky lymph, when incubated with samples of either lymph or plasma, has been used in these experiments as assay for clearing activity. No determinations were made of changes in free fatty acids or neutral fat in the incubation mixture. Others have shown however, that *in vitro* decrease in optical density, or clearing, comes about through formation of soluble fatty acid-protein complexes with concomitant reduction in triglycerides(10).

Summary. *In vitro* clearing activity has been measured in samples of human plasma and thoracic duct lymph before and after heparin administration. When administered in amounts sufficient to cause lipid clearing activity in plasma, heparin does not produce clearing activity in thoracic duct lymph.

1. Anderson, N. G., Fawcett, B., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 768.
2. Grossman, M. I., *J. Lab. and Clin. Med.*, 1954, v43, 445.
3. Hollet, C., Cole, W. E., Meng, H. C., *Fed. Proc.*, 1953, v12, 70.
4. Linder, E., Bloomstrand, R., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 653.
5. Grossman, M. I., *J. Lab. & Clin. Med.*, 1954, v43, 445.
6. French, J. E., Robinson, D. S., Florey, H. W., *Quart. J. Exp. Physiol.*, 1953, v38, 101.
7. Young, W., Freeman, N. K., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 463.
8. Robinson, D. S., Jeffries, G. H., French, J. E., *Quart. J. Exp. Physiol.*, 1954, v39, 165.
9. Baker, S. P., Levine, H., Turner, L., Dubin, A., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 670.
10. Shore, B., Nicholas, A. V., Freeman, N. K., *ibid.*, 1953, v83, 216.

Received March 11, 1960. P.S.E.B.M., 1960, v104

Depot Fat Mobilization in Spinal Cord Sectioned and Restrained Rats.* (25751)

ALAN C. LEVY AND ESTELLE R. RAMEY (Introduced by John C. Rose)
Depts. of Physiology, Howard University School of Medicine and Georgetown University School of Medicine, Washington, D. C.

The work of Goering(1), Wertheimer(2), Hausberger(3), Boecke(4) and others supports the concept that metabolism of adipose tissue is influenced by neural factors. Thus, autonomic nervous system may alter movement of fat to and from storage depots(3, 5-8). Our previous work showed that certain autonomic blocking agents prevented mobilization of fat from epididymal depots of adrenalectomized rats during fast. Surgical intervention within the nervous system eliminates ambiguities of the mechanism of blocking drug action. Therefore, spinal cord section was used as alternative tool. Since paralysis induced by this procedure alters activity of animal and the energy expended, restraining cages were used to immobilize equally both control and cord sectioned animals.

Methods. Male rats of Holtzman strain, weighing 90-110 g, were used. The epididymal fat pad was utilized as index of peripheral depot mobilization. Fat body removal was performed as previously described(9). Total lipids were extracted and weighed according to Bloor's method(10). The first fat depot was removed just prior to beginning of 24-hour fast. The second pad was excised upon termination of starvation. The difference between amount of extracted fat in the 2 epididymal depots determined extent of fat mobilization. Initial depot removal was altered from side to side throughout experiments. All spinal cord sections and sham operations were performed under ether anesthesia. A midline incision was made over region of thoracic vertebrae 2-4. Laminectomy was performed and spinal cord exposed, which was then severed. Muscles were sutured and skin closed with wound clips. Only those animals with complete cord transection, as determined on autopsy, were used. Sham operations consisted of cord transection procedure without actual cutting of the spinal cord. Fasting began with end of surgery. Restraint was accomplished by confining the rat within a wire screen cylinder. This prevented most bodily activity and allowed only slight movement of head. Restraint immedi-

* This investigation made with assistance of grant from Committee on Research, Council on Drugs, A.M.A. and from Eugene and Agnes Meyer Research Fn.