Magnesium concentration of the tissues varied considerably from dog to dog. Muscle tissues, either from heart, skeletal muscle, or gastrointestinal tract had the most constant concentration, standard deviations being less than 5-20% of the means among different dogs. Brain and glandular organs of the gastrointestinal tract had about the same degree of variations in magnesium concentration. This magnitude of variation was also observed in plasma concentration among human beings(2). The magnitude of the variations was not decreased when the values were expressed in dry weight thus indicating that they were not the result of differences in water content of the tissues. Those tissues containing a large proportion of collagenous material or connective tissue varied much more in magnesium concentration among the dogs. The reason is not known. The greatest variation observed in bone marrow and cartilage might be due to varying degrees of calcification as bony structures contain disproportionally larger amounts of magnesium.

The concentrations of magnesium in tissues obtained in this study were generally higher than those reported by chemical methods (Table II). Since reproducibility and recovery were both quite satisfactory with this method, the data reported in the present study were considered more accurate.

Summary. The content of magnesium in 73 different tissues and body fluids of the dog was determined by flame photometry. The heart, skeletal muscle, glands, veins, epididymis, and cornea had the highest concentrations of magnesium among the soft tissue. Bone had the highest concentration of magnesium among all tissues examined. Collagenous tissue manifested the greatest variations among the dogs. The values obtained by this method were higher than those previously reported for chemical methods. The procedure of acid digestion is satisfactory for flamephotometric determination of magnesium in tissue and the flamephotometric method is sufficiently simple and accurate for general application.

1. MacIntyre, I., Flame photometry. Advances in Clinical Chemistry, 1961, v4, 1.

2. Alcock, N., MacIntyre, I., Radde, I., J. Clin. Path., 1960, v13, 506.

3. Spector, W. S., Handbook of Biological Data, W. B. Saunders Co., Philadelphia & London, 1956.

4. Eichelberger, L., McLean, F. C., J. Biol. Chem., 1942, v142, 467.

5. Eichelberger, L., Kollros, J. J., Walker, A. E., Roma, M., Am. J. Physiol., 1949, v156, 129.

6. Eichelberger, L., Bibler, W. G., J. Biol. Chem., 1940, v132, 645.

7. Widdowson, E. M., Dickerson, J. W. T., Chemical composition of the body in Mineral Metabolism, Academic Press, New York & London, 1964, vII B, p1.

8. Eichelberger, L., Eisele, W. C., Wertzler, D., J. Biol. Chem., 1943, v151, 177.

Received November 19, 1964. P.S.E.B.M., 1965, v118.

Effect of Thymectomy on Hamsters.* (29911)

ROBERT A. ROOSA, DARCY B. WILSON† AND V. DEFENDI‡
(Introduced by H. Koprowski)
Wistar Institute of Anatomy and Biology, Philadelphia, Pa.

Neonatal thymectomy in several animal species produces a generalized impairment of immunological responses that is manifested by a depression both of circulating antibody levels after antigenic stimulation and of cell mediated hypersensitivities(1,2,3,4). Correspondingly, there is a decrease of circulating lymphocytes and atrophy of lymphoid follicles in the spleen and lymph nodes. In addition, several laboratories have reported the occurrence of a fatal wasting disease in newborn thymectomized animals. The incidence of this disease varies considerably, depending

* This work was supported in part by Am. Cancer Soc. Grant E-89F and USPHS Grant CA04534 NCI.

† Helen Hay Whitney Foundation Fellow.

‡ Leukemia Society Scholar.

upon the strain(5,6,7) and the species tested (8,9,10,11).

Thymectomized animals, because of their impairment of immunological responses, represent a useful tool for the study of tumor induction by viral and chemical agents, particularly where these tumors are antigenic to the autochthonous host(12). The increased susceptibility of thymectomized hamsters to tumor induction by polyoma virus has been reported(11,13).

This report concerns the effect of thymectomy at various ages in hamsters, with emphasis on growth rate, incidence of wasting disease and responses to skin homografts in these animals.

Materials and methods. The animals used for thymectomy in this study were the outbred Syrian hamster *(Mesocricetus auratus)* obtained from the Lakeview Hamster Colony, Newfield, N. J. Pregnant females were received at approximately the 8th day before parturition, and the thymus was removed from the infants at various times from within minutes until 3 months after birth.

Thymectomies were performed on the infants after they had been chilled at 4°C. Older animals were anesthetized with chloral hydrate (0.36 mg/g body weight). After the animals were secured, back down, on a contoured sponge rubber pad and the thorax swabbed with alcohol, an incision was made in the skin over the thymic area. After reflecting the skin from the anterior thoracic region a piece of sternum from the apex to the third rib was removed, and the thymus gently lifted out with a glass pipette connected to a suction pump. The wound was then sprayed with a fine powder of antibiotics§ before 3 to 4 interrupted sutures were used to close the skin edges. The incision was then sprayed with a wound dressing.‖ Sterile instruments were employed throughout the operative procedure. Control littermates were sham-thymectomized by the same operative procedure with the exception that the thymus was left intact. Within each litter most of the animals were thymectomized, the remainder were either sham-thymectomized or not operated upon. Marking of the animals was accomplished with an electric cauterizer.¶ After the operation the animals were warmed to 37°C before being returned to their mother. The number of animals returned never exceeded ten.

Food consisted of Purina lab chow, wheat germ flakes, rolled oats, fresh kale and water *ad libitum.* A broad spectrum antibiotic,** at a concentration of 0.25 mg/ml, was added routinely to the water 5 days per week during the first 2 weeks.

The animals were weighed each week and dead animals were carefully scrutinized for a residual thymus. Fragments of thymus were observed in only a few cases and confirmed by histological section, in which instance they were not included in the data.

Skin grafts. Skin from female hamsters of the isogenic CB strain were applied orthotopically to approximately 2- to 3-month-old Lakeview Hamsters according to the methods of Billingham(14). The bandages were usually removed on the 9th day; assessments of degree of graft survival were made daily until the 20th day, thereafter 3 times a week.

X-irradiation. Animals, which were thymectomized or sham-operated at various times after the day of birth, were irradiated with 350 r 14 days later along with their non-operated littermates. For irradiation, the animals were enclosed in a lucite chamber and then placed on a rotating table under an irradiation source from a Keleket X-ray unit (200 Kv and 20 ma); no filter was used and the distance was 40 cm; a Victoreen Radocon ratemeter was employed to calculate the dosage.

Results. Hamsters thymectomized as newborns. During the period immediately following the operation the majority of hamsters did not show changes of their normal feeding habits nor impairment of mobility. In this respect they were indistinguishable from their non-operated littermates. The wound healed very rapidly with no suppurations.

The well-being of the thymectomized hamsters is reflected in the growth curve illus-

§ Powdalator-es, Abbott Labs.

‖ Scan, Johnson and Johnson.

¶ National Elec. Inst. Co., Elmhurst, L. I., N. Y.

** Tetracycline-HCl, American Cyanamid Co.,

trated in Fig. 1, which comprises a consecutive series of litters weighed weekly for a period of 16 weeks. The weight of the thymectomized animals lags only slightly as compared with both of the control groups. At the 14th week, the slight difference in mean

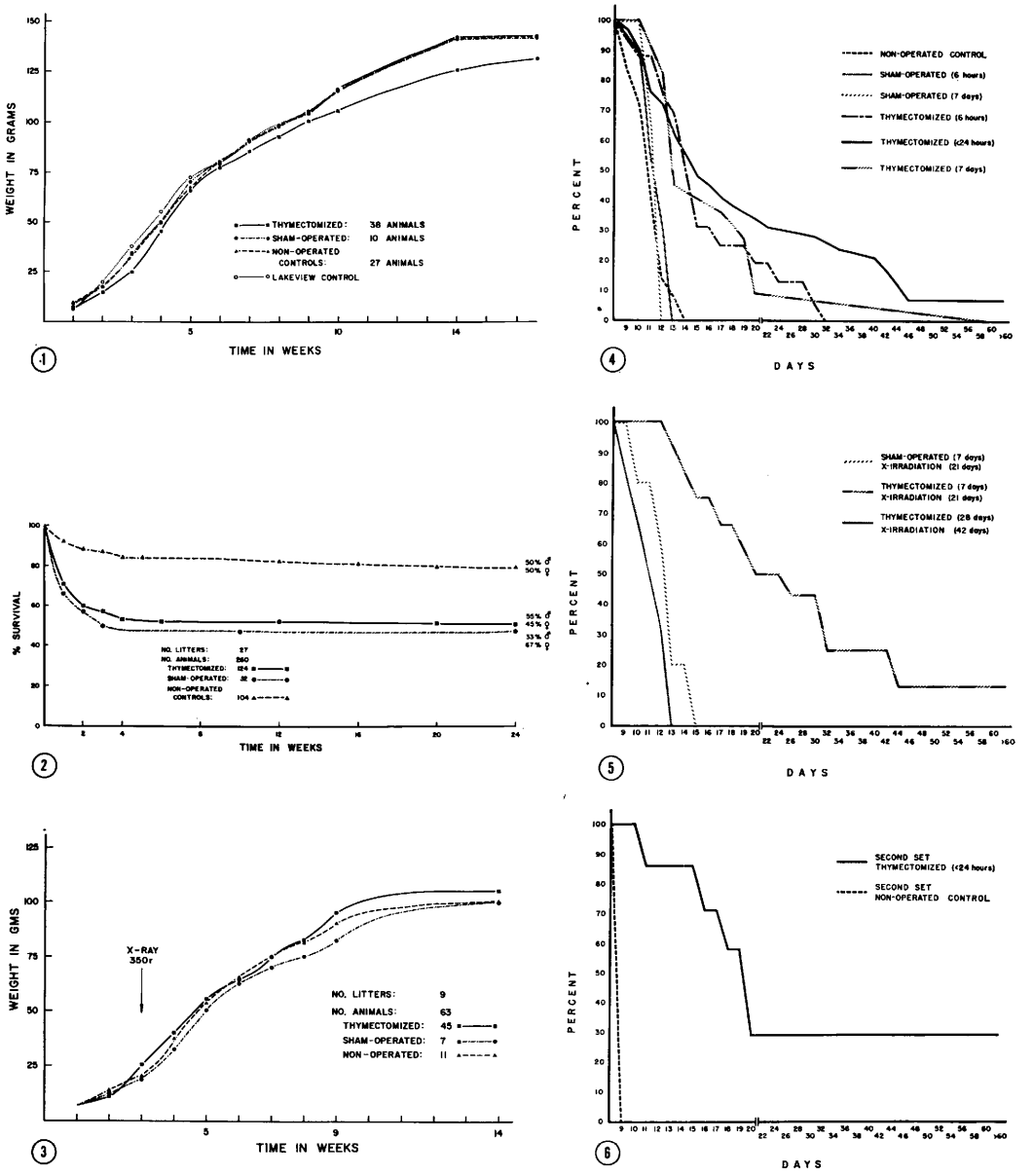

FIG. 1. Average weights (g) of Syrian hamsters neonatally thymectomized, sham-operated and their non-operated littermates.

FIG. 2. Percent survival of Syrian hamsters thymectomized and sham-operated within 24 hr after birth and sex comparison of surviving animals.

FIG. 3. Average weights (g) of X-irradiated Syrian hamsters thymectomized and sham-operated at 7 days and their non-operated littermates.

FIG. 4. Cumulative percent survival of skin homografts to hamsters thymectomized at birth or at 7 days.

FIG. 5. Cumulative percent survival of skin homografts to hamsters which were X-irradiated and thymectomized or sham-operated.

FIG. 6. Second set skin homografts to thymectomized and non-operated controls.

TABLE I. Per Cent Survival of X-Irradiated* Syrian Hamsters Thymectomized and Sham-Operated at 7 Days and Their Non-Operated Littermates.

	No. animals	Time in weeks 3	4	5	7	Months 6
		(%)	(%)	(%)	(%)	(%)
Thymectomized	45	98	96	93	91	91
Sham-operated	7	100	100	100	100	100
Non-operated	11	100	100	100	100	100

* 350r at day 21.

weight between thymectomized and control animals was shown by a t test to be not significant ($0.4>p>0.3$). The mean weights of control groups did not differ significantly from that of the Lakeview golden hamster (60 males and 60 females, randomly selected) as compiled by the breeder at his farm. About 10% of the thymectomized and sham-operated hamsters showed mild manifestations of wasting disease (ruffled hair, underweight) from which they eventually recovered.

Survival studies for a group of 27 consecutively thymectomized litters are represented in Fig. 2. This figure represents the per cent survivors for 260 animals divided into 3 groups: 124 thymectomized, 32 sham-operated, and 104 non-operated. No litters were discarded from these computations. The largest loss of animals in each of the 3 groups occurred during the first week, and this was due to post-operative mortality and to cannibalism by the mother. The data show that thymectomized and sham-operated animals die and/or are cannibalized during this period at about an equal rate.

Between the second and fourth weeks, additional deaths occurred in all 3 groups, although at a lower incidence in the non-operated control group. Dying animals were usually smaller with some loss of body fat and occasional animals showed signs of diarrhea with enlarged cecum. Usually, entire litters showed signs of wasting. From the fifth week on very few deaths occurred and rate of death in the thymectomized group was not greater than in either of the control groups. These results indicate that more than 50% of hamsters thymectomized on the first day of birth survived for a period of 6 months. There was no evidence that sex was a factor in the death rate. A considerable number of thymectomized hamsters (2-3 months old) died within one week following skin homografts indicating that these animals are not able to withstand an operative procedure that normally does not affect the well-being of control animals. After 6 months most of the litters were utilized for experimental purposes. A representative number were observed without further treatment for at least 1½ years with no intercurring deaths.

Hamsters thymectomized at seven days. In an attempt to determine what effect the age at thymectomy might have on survival and immune response a group of hamsters was thymectomized at various times after birth. Previous reports(6) have demonstrated that survival of skin homografts was extended if mice thymectomized as adults were also X-irradiated. A group of hamsters thymectomized at 7 days of age and in addition irradiated with 350 r at 21 days of age was observed for survival and growth studies. Table I demonstrates that this schedule produces more survivors than when the operation was performed on newborn animals. No wasting disease occurred in this group. In the group of 9 litters shown here none of the controls were lost while only a single thymectomized animal died before irradiation and the mortality to 6 months of age was only 9%.

Animals were weighed at weekly intervals and Fig. 3 shows that there is very little spread between the various groups. Average weights of each of the groups were slightly lower than those shown in Fig. 1. It is likely that the irradiation dose given at the third week accounts for the lower weights in all these animals.

Studies of skin homografts in thymectomized hamsters. Skin homografts from female CB hamsters were transplanted to members of each of 4 groups of thymectomized hamsters (thymectomy at 6 hours, 24 hours, 7 days and 28 days); to members of 4 groups of sham-operated animals and onto an additional group of non-operated hamsters. All primary grafts were applied to 2-3-month-old animals. The day that no surviving epithelium could be seen by gross inspection was

TABLE II. Median Graft Survival Times Estimated from Graft Survival Curves.

Group	Treatment	No. of animals	M.S.T.
A	Control, non-operated	36	10.5
	Thymectomized, <24 hrs	29	14.8
	Sham-operated, <6 hrs	9	11.2
	Thymectomized, <6 hrs	16	14.0
	Sham-operated, 7 days	3	11.2
	Thymectomized, 7 days	11	13.0
B	Sham-operated, 7 days X-irradiated, 21 days	5	12.2
	Thymectomized, 7 days X-irradiated, 21 days	12	22.0
	Thymectomized, 28 days X-irradiated, 42 days	6	11.0
C	Control, non-operated (second set)	6	8.5
	Thymectomized, <24 hrs (second set)	7	19.2

considered to be the last day of rejection. The results of this experiment are shown in terms of graft survival times in Fig. 4, 5 and 6. From the data presented in these Figures median survival time (MST) of the skin grafts is computed and summarized in Table II. Whereas the median survival time of CB skin grafts on non-operated control hamsters was 10.5 days, the survival time of similar grafts on hamsters thymectomized at times up to 24 hours of age was 14.8 days. Thymectomy within 6 hours after birth did not result in further prolongation of graft survival. Thymectomy at 7 days extended the MST of the grafts but was not as effective as thymectomy on newborn animals. Survival times of skin grafts on hamsters comprising the sham-operated groups was similar to that of the non-operated control group. Fig. 5 shows the result of skin grafting to hosts which were thymectomized at 7 and 28 days after birth and irradiated 2 weeks later. The combination of thymectomy at 7 days and irradiation at 21 days greatly extended the graft survival time (MST = 22 days) whereas grafts applied to animals thymectomized at 28 days and irradiated 14 days later did not survive longer than the controls (Fig. 5, Table II).

To determine what effect thymectomy might have on rejection time of second-set skin homografts, thymectomized hamsters which had sustained their first skin homograft in good condition for a period greater than 20 days were regrafted with CB skin 60 days later. Survival times of these grafts were compared with second-set survival times of grafts made to the non-operated control group (Fig. 6). Thymectomy not only significantly prolonged survival time of first-set homografts (Fig. 4) but totally abolished any second-set reactivity (Fig. 6).

Discussion. These data support and extend our previous findings concerning the fate of thymectomized hamsters(9,11). The incidence of wasting in our thymectomized group of some 400 animals did not exceed 10% and no differences in regard to sex incidence were recorded.

The largest group of animals was operated on at less than 24 hours after birth; while these thymectomized animals were slightly smaller than their non-operated littermates, only a few animals could be considered as wasted. The incidence of wasted animals was distributed through all 3 groups (thymectomized, sham-operated and non-operated controls). The spread of animal size was such that within a litter the thymectomized animals could be recognized only by identifying the animal's number.

Mortality was almost completely abolished when thymectomy was performed at one week of age; the size and weights of animals were again similar to their littermate controls. It should also be pointed out that these animals were also subjected to an X-irradiation dose of 350 r 2 weeks after the thymus was removed.

Our results on the incidence of wasting disease are in marked contrast to those reported by another laboratory where wasting was observed in 63% of the males thymectomized between 1-4 weeks of age(10). One can only speculate as to the cause of this discrepancy concerning the effect of thymectomy in the golden Syrian hamster. It is well known that there is a strain difference in mice with regard to susceptibility to wasting after thymectomy(5,6,7). A similar difference could exist between the Syrian hamsters used in other studies(10) and those reported here. Infections in animals have often been speculated upon as causative agents in wasting

but, to the best of our knowledge, no such agent has been reported. A hepatotrophic virus has been recovered from wasting mice but it is not thought to be primarily responsible for their death(15). Azar(16) in a study on neonatally thymectomized rats demonstrated that animals treated with antibiotics did not develop a wasting syndrome, while untreated animals did. Also, Wilson (17) reported that thymectomy in axenic mice did not result in wasting, but animals exhibited wasting symptoms when they were thymectomized in germ-free conditions and then were removed to a normal environment.

In this study, we have noted that diarrhea is associated with animals that waste, and most often wasting of an animal is followed by the entire litter becoming similarly affected(9,11). As previously mentioned, our animals were operated upon with sterile instruments in an aseptic field, with prophylactic doses of a wide spectrum antibiotic being routinely administered to all litters.

Numerous studies, predominantly in mice, have shown, in general, that the thymus is essential for development of the immunological response mechanism during ontogeny(18). The present studies show that immune mechanisms, as measured by capacity to reject foreign skin grafts, are debilitated in hamsters as a consequence of thymectomy at, or shortly after, birth. Unlike the thymectomized mouse, in which the rejection of homologous and even heterologous skin grafts is greatly delayed(2), similarly treated hamsters show only a moderate prolongation of homograft survival. Fifty per cent of the grafts are rejected by the 15th day and more than 90% of the animals reject their grafts by the 46th post-operative day. On the basis of a shorter gestation period, one might have incorrectly speculated that the hamster (16 days) would be immunologically less mature at birth than the mouse (20 days).

When hamsters are thymectomized at the 4th week after birth and irradiated 2 weeks later they are able to reject skin homografts in a normal manner. This is in contrast to mice which, when thymectomized and irradiated in adult life (12 weeks), show an impairment of responsiveness to foreign skin homografts(6). These differences could be attributed to the fact that the depression of the lymphoid system in the thymectomized hamster is less marked than in thymectomized mice(11).

It is of interest that skin homografts survived longest in animals thymectomized at 7 days of age and irradiated 2 weeks later. These animals, then, appear to be the most suitable for further experimental treatment since their mortality is practically nil.

Summary. The effect of thymectomy at various ages on Syrian hamsters has been investigated. Forty-four per cent of the animals thymectomized and sham-operated at less than 24 hours did not survive the first 3 post-operative weeks. The remaining animals, however, appeared as healthy and survived as long as their control littermates. A mild wasting disease occurred in 10% of the thymectomized and sham-operated animals. The incidence of wasting disease and of lethality was equally distributed in the 2 sexes. Survival of skin homografts was extended in animals thymectomized at less than 24 hours (MST = 14.8 days in contrast to 10.5 days in the controls). Operative mortality and cannibalism were greatly reduced and wasting disease did not occur in animals thymectomized at one week of age and X-irradiated 2 weeks later. In these animals skin homografts survived longer than in any other group in this study (MST = 22 days).

The authors wish to thank Miss M. Westenius, Miss M. Lindstrom, Mrs. E. Bailey and Mr. M. Davis for excellent technical assistance.

1. Miller, J. F. A. P., Lancet, 1961, vII, 748.
2. ———, Proc. Roy. Soc., B, 1962, v156, 415.
3. Arnason, B. G., Jankovic, B. D., Waksman, B. H., Wennersten, C. K., J. Exp. Med., 1962, v116, 177.
4. Good, R. A., Dalmasso, A. P., Martinez, C., Archer, O. K., Pierce, J. C., Papermaster, B. W., ibid., 1962, v116, 773.
5. Parrott, D. M. V., Transpl. Bull., 1962, v29, 102.
6. Miller, J. F. A. P., Lancet, 1963, v1, 44.
7. Law, L. D., Dunn, T. B., Trainin, N., Levey, R. H., The Thymus, Wistar Inst. Symposium No. 2, 1964, (V. Defendi, D. Metcalf, Ed.), Wistar Inst. Press, Philadelphia, p105.
8. Jankovic, B. D., Waksman, B. H., Arnason, B. G., J. Exp. Med., 1962, v116, 159.

9. Roosa, R. A., Wilson, D., Defendi, V., Fed. Proc., 1963, v22, 599.

10. Sherman, J. D., Dameshek, W., Nature, 1963, v197, 649.

11. Defendi, V., Roosa, R. A., Koprowski, H., Thymus in Immunobiology, R. A. Good, A. E. Gabrielsen, Ed., Hoeber-Harper, New York, 1964, p504.

12. Defendi, V., Roosa, R. A., The Thymus, Wistar Inst. Symposium Monograph No. 2, 1964, (V. Defendi, D. Metcalf, Ed.), Wistar Inst. Press, Philadelphia, p121.

13. ———, Fed. Proc., 1964, v23, 393.

14. Billingham, R. E., Transplantation of Tissues and Cells, R. E. Billingham, W. K. Silvers, Ed., Wistar Inst. Press, Philadelphia, 1961, p1.

15. East, J., Parrott, D. M. V., Chesterman, F. C., J. Exp. Med., 1963, v118, 1069.

16. Azar, H. A., Williams, J., Takatsuki, K., The Thymus, Wistar Inst. Symposium No. 2, 1964, (V. Defendi & D. Metcalf, Ed.), Wistar Inst. Press, Philadelphia, p75.

17. Wilson, R., Sjodin, K., Bealmar, M., ibid., p89.

18. Miller, J. F. A. P., Science, 1964, v144, 1544.

Received November 13, 1964. P.S.E.B.M., 1965, v118.

A Method for Determination of Plasma Free Fatty Acids.* (29912)

PETER D. S. WOOD AND H. S. SODHI† (Introduced by Laurance W. Kinsell)
Institute for Metabolic Research, Highland-Almeda County Hospital, Oakland, Calif.

It has been shown that carboxylic acids may be quantitatively converted to their C^{14}-methyl esters by treatment under mild conditions with an ethereal solution of C^{14}-diazomethane(1). We present here a simpler method for isolation and quantitation of C^{14}-methyl esters derived from plasma FFA.

Methods and results. Lipids were extracted from plasma and washed using the method of Folch *et al*(2); water containing 0.04% (w/v) calcium chloride was used as wash solution to prevent loss of FFA at this stage (3). Six ml of an ethereal solution of C^{14}-diazomethane was prepared from 1 g of N-methyl-N-nitroso-*p*-toluene sulfonamide (New England Nuclear Corp.) containing about 25 μc of radioactivity. The solution of C^{14}-diazomethane was stored in a closed container in a freezer and used within 2 days of preparation; transfer to reaction tubes was made with a pre-chilled pipette to minimize evolution of gas. All operations with diazomethane were conducted in a fume hood. Columns of silicic acid (Bio-Rad 325 mesh, activated at 110° for 3 hours) were prepared and washed thoroughly with petroleum ether, b.p. 30-60° (PE) in glass tubes (200 × 8 mm).

For estimation of plasma FFA, the lipid extracts from plasma (0.1-0.5 ml) were evaporated to dryness under nitrogen in a 10 ml glass-stoppered centrifuge tube, and immediately redissolved in one drop of methanol. Ten drops of the fresh ethereal solution of C^{14}-diazomethane were added, the tubes stoppered, mixed and placed in the dark at room temperature for 30 minutes, with swirling after 15 minutes. The solvents and excess diazomethane were evaporated under nitrogen, and the residues transferred quantitatively with a total of 12 ml of PE to 3-cm columns of silicic acid. The PE emerging from the columns was discarded, and elution with 20 ml of 2% (v/v) diethyl ether in PE was commenced at once. The whole of the eluate was collected in 20 ml counting vials, the solvent evaporated and the residue dissolved in scintillation fluid and counted to give a standard deviation of less than 2%.

Known amounts (usually 25-250 μg) of pure palmitic or stearic acid were treated as described above and from the results a standard curve of radioactivity *vs* weight of FFA was constructed.

A blank determination was also carried through, in which the C^{14}-diazomethane was added to a tube containing one drop of methanol only. The count from this tube

* Supported in part by U.S.P.H.S. Grant.

† Present address: Stanford Univ. Med. Center, Palo Alto, Calif.