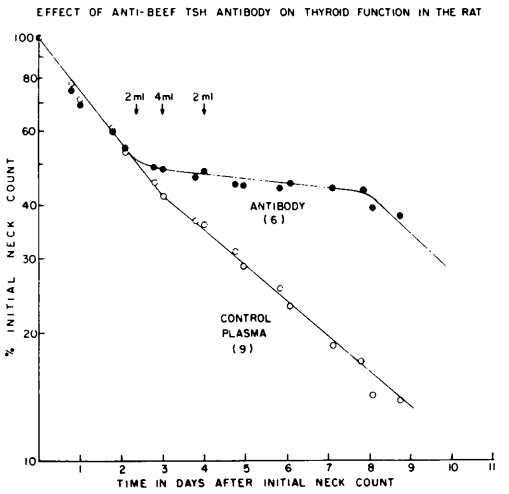

FIG. 1. Effect of anti-beef TSH antibody (rabbit) on thyroid function in the rat. Illustrated here is the course of thyroidal I^{131} release as measured by external neck counting in groups of rats. The marked inhibitory effects of antibody injections are contrasted with the minimal effects due to normal rabbit plasma. The curve has been drawn to suggest that return to normal thyroid function has begun by the eighth day. There are insufficient data however to establish this point with certainty, and it is possible that the last 2 determinations in the antibody treated group fall on a straight line drawn between the third and ninth day.

bit antibody to beef TSH. Cross reactivity of the antibody, recognized by a number of workers(5-7) is thus confirmed. The material is potent enough to inhibit endogenous TSH action. A similar effect on endogenous thyroid function in the rat has also been described by Baschieri *et al.*(8) who showed that injection of 0.1 ml of immune rabbit plasma every other day for 10 days led to significant inhibition of thyroid I^{131} uptake and to decrease in thyroid gland size. The minimum effective dose of the anti-plasma has not been determined; following *in vitro* incubation 0.005 of antiplasma effectively neutralizes 1.0 mU of USP standard TSH as determined by bioassay in the mouse.

Of particular interest is the time course of the thyroid response to the anti-plasma injection. It closely resembles that seen following thyroxine injection in the rabbit(9) and the rat(10), as well as that produced by hypophysectomy in these species(10,11). These results further confirm the fact that the thyroid mechanisms responsible for release of thyroid hormone from the gland are dependent from minute to minute upon the local concentration of thyrotrophic hormone.

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Test for Metabolic Attack on Tris(Hydroxymethyl)Aminomethane and α-Hydroxymethylserine in the Rat.* (27728)

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Tris(hydroxymethyl)aminomethane† is under investigation as a therapeutic alkalinizing agent (see bibliography in reference 1). The fact that this substance has appreciable affinity for liver alcohol dehydrogenase, and is apparently slowly oxidized by it(2), raises

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† Tris is used here for tris(hydroxymethyl)aminomethane.

the possibility that some metabolic oxidation of it may occur. The first product expected from this oxidation, through conversion of one of the 3 hydroxymethyl groups to a carboxyl group, would be α-hydroxymethylserine, which we prepared several years ago by the permanganate oxidation of tris(3). If this amino acid were formed, it would probably survive, since we have previously shown that it, along with α-methylserine, largely escapes metabolic attack in the rat, although it is taken up into liver and muscle(3). Wilson and Snell recently have obtained a bacterial enzyme that attacks both amino acids(4).

The present study failed to show an observable quantity of any other radioactive substance in the urine after injecting labeled α-hydroxymethylserine; in the first day 96% of the urinary radioactivity was obtained chromatographically in portions of eluate characteristically containing the unchanged amino acid. We have looked for the labeling of carrier quantities of α-hydroxymethylserine added to the urine of rats receiving tris labeled in the hydroxymethyl groups. The α-hydroxymethylserine gained no significant radioactivity.

Experimental. Tris(hydroxymethyl-C^{14}) aminomethane was purchased from the New England Nuclear Corp. and used without purification. Either 0.23 mg or 2.25 mg of this preparation (one or 10 microcuries) in 1.0 ml of 0.9% NaCl solution was injected subcutaneously in the scapular region into female rats weighing from 50 to 70 g. The animals were placed on a raised plastic shelf in a bottle, and fresh air passed through the container. The effluent air was bubbled through Hyamine hydroxide solution to absorb CO_2, as previously described(5). This collection was made during the first 6 hours and from the 24th to the 28th hour. Urine samples were collected representing the first, second and third 12-hour periods. The animals were provided with water but no food during the 36 hours of study. Blood was collected from the tail at 24 and 36 hours. α-Hydroxymethylserine, labeled in all carbon atoms except the α-carbon, was prepared by permanganate oxidation of the labeled tris, using a micro adaption of the usual procedure(3). This substance was injected in quantities of 3.6 mg, representing 1.1 microcuries.

For chromatography a quantity of urine representing about 150,000 cpm was taken. If tris had been injected 50 mg of α-hydroxymethylserine was dissolved in the sample. The urine was adjusted to pH 3.0. A resin column was prepared using Dowex-50-8X that had been washed in turn with 4*N* HCl, water, and 4*N* aqueous ammonia. It was then treated repeatedly with 0.2 *M* ammonium formate, pH 3.0, until the pH remained at this value. The urine sample was then applied, and the same ammonium formate solution used to carry the sample into the column and for selective elution. Successive samples of 13.5 ml of eluate were collected. α-Hydroxymethylserine appeared in a characteristic peak within the range of 300 to 420 ml. Serine appeared between 400 and 500 ml. No elution of tris was observed during the passage of 1300 ml of buffer through the column.

Urine samples were also applied to filter paper and subjected to ascending chromatography using t-butanol, formic acid and water in the proportions 70:15:15.

The radioactivity of plasma and urine, and of Hyamine hydroxide solutions, was determined by liquid scintillation counting(5) as described previously.

Results. Fig. 1 shows the radioactivity of 12 successive portions of eluate of urine after injection of labeled α-hydroxymethylserine. The urine sample represented the second 12 hours after the injection. Ninety-six percent of the counts found in the whole urine are collected in these 12 samples. The position of the elution peak is characteristic of α-hydroxymethylserine. A similar result was obtained for the urine sample represented by the first 12 hours. From 51 to 99% of the injected radioactivity appeared in the urine within 24 hours. Nevertheless, significant amounts of volatile C^{14}, presumably $C^{14}O_2$, were trapped meanwhile from the atmospheric environment of the rat (Table I). Most of the excretion by this route was distinctly retarded in relation to excretion into the urine, a result to be expected if a small part of the C^{14} were removed as a fragment

(*e.g.*, the formyl group) that subsequently becomes part of other metabolites. Schirch and Mason (personal communication) have recently observed in a preliminary manner that a purified serine transhydroxymethylase of rabbit liver attacks α-methylserine, although no attack on α-hydroxymethylserine was observed.

Fig. 2 shows that no significant radioactivity appears in the urine in the region characteristic of α-hydroxymethylserine during the first 12 hours after injection of tris-C^{14}. If the radoactivity of each of these samples were really higher than shown in the Figure by one standard deviation of the mean, the total radioactivity excreted in these samples would still represent only about 0.1% of that injected. Similar results were obtained for the subsequent two 12-hour intervals, and also when 10 rather than one microcurie was injected. No radioactive substance other than the injected compound could be detected in either case on paper chromatograms.

The radioactive carbon excreted into the atmosphere represented 0.5% and 0.7% of that present in the tris preparation injected. This radioactivity appeared largely in the first 2 hours. Since only about half of the tris was excreted into the urine in the first 12 hours, the small initial release of volatile C^{14} was largely terminated while most of the tris was still present in the animal. Hence it probably was largely due not to tris itself but to some radioactive contaminant in the preparation. In a typical experiment, 52% of the tris appeared in the urine in the first, 10% in the second and 4.5% in the third 12-hour interval (cf., 6).

Summary. We may conclude that tris undergoes very little if any metabolic oxidation in the rat, if we agree that α-hydroxymethylserine must be an intermediate in that oxidation. This conclusion follows from the finding that no significant quantity of radioactive α-hydroxymethylserine appears in the urine after injection of labeled tris, whereas when the amino acid is injected it appears largely in the unchanged form in the urine, although a part is excreted into the atmosphere, presumably as $C^{14}O_2$.

TABLE I. Appearance of Volatile C^{14} after Injection of Labeled α-Hydroxymethylserine into the Rat. Excretion for the sixth to the 24th hr was crudely estimated by linear interpolation. Assays at intermediate points indicated that excretion had passed its peak at 24 hr.

	cpm volatilized per hr				
	Hr				Estimated % of dose volatilized in 28 hr
cpm inj.	1	2	3-6	24-28	
827,000	315	185	525	1660	3.5
1,760,000	1560	630	910	2930	2.9

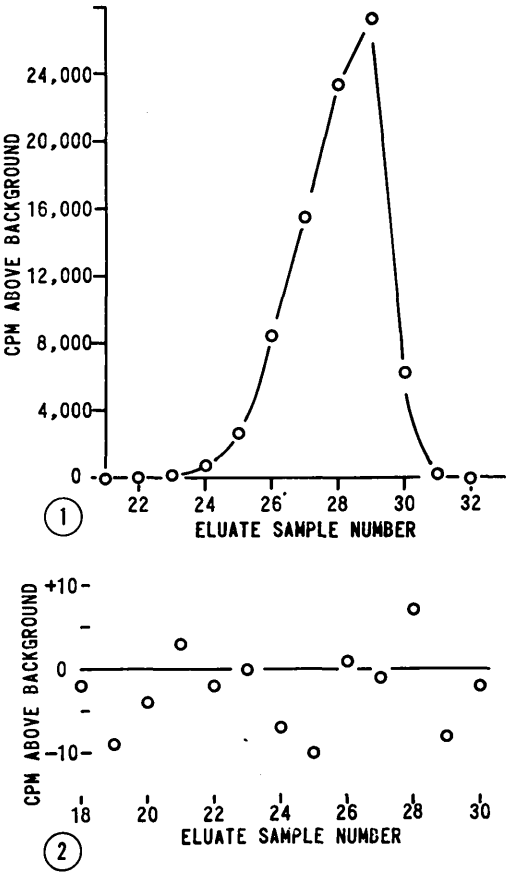

FIG. 1. Elution from Dowex-50 of the only radioactive component observed in urine after injecting labeled α-hydroxymethylserine. The amino acid added to urine *in vitro* was eluted in the same range of eluate volume.

FIG. 2. Failure of labeled radioactive α-hydroxymethylserine to appear on the urine chromatogram, after injection of labeled tris. The points show variation in radioactive counts in relationship to background. Compare with Fig. 1.

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Further Studies of Suppression of the Homograft Reaction by Thymectomy in the Mouse.* (27729)

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Surgical ablation of the thymus is a valuable approach to demonstration of the role of this gland in the immune response in several species of mammals. The immunologic-depressive effect of thymectomy appears to be a function of the age at which the thymus is removed and of the difference in immunological maturation at birth in the species studied (1-4). In experiments to ascertain the inhibiting effect of neonatal thymectomy in development of the capacity to reject skin homografts it was initially observed(5,6) that prolongation of skin graft survival was produced only in strains of mice sharing the H-2 histocompatibility locus and homologous with regard to other genetic loci controlling "weaker" factors. Subsequently we established that complete thymectomy performed in mice immediately after birth allows acceptance and growth of tumor tissue originally developed in a mouse differing at the H-2 histocompatibility locus(7,8). These results together with the virtually complete inhibition of immunological response to the bacteriophage T_2 in mice completely thymectomized at birth and the demonstration that small remnants of thymus tissue permitted an appreciable antibody synthesis(4) prompted us to repeat the skin homotransplantation studies in thymectomized mice and to evaluate their immunological response when confronted with normal homologous tissues possessing a pronounced difference in antigenic constitution. In the present report we demonstrate that complete thymectomy in neonatal mice produces sufficient inhibition of immunological maturation to permit acceptance of skin homografts even when the antigenic disparity between the strains of animals studied is conditioned by the H-2 histocompatibility locus. These experiments also demonstrate that when thymectomy is carried out in weanling mice no effect on skin homograft survival is demonstrable if the strains of mice differ at the H-2 histocompatibility locus whereas prolonged survival of skin homografts is sometimes observed when the homologous mice share the antigens controlled by H-2 locus. Finally it is shown that complete thymectomy at birth permits female mice of the C57Bl strain to accept at a later date skin isografts from male donors, thus establishing that neonatal thymectomy permits them to overcome the Eichwald-Silmser(9) histocompatibility barrier.

Material and methods. Inbred mice of the C57Bl/1, DBA/2 and C3H/Bi strains and F1 hybrids resulting from the cross between Balb/C and DBA/2 and between A and C3H strains were employed. Groups of mice of the C57Bl/1, DBA/2 and C3H strains were thymectomized or sham-operated either at birth or 35 days later. At this later age, groups were also prepared in which both thymus and spleen were removed. The technic for thymectomy in newborn mice was essentially the same as that described by Dischler and Rudali(10) and the procedure employed in older animals was the same as that previ-

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