hibiting the oxidation of I^- to I_2 or (b) by reacting with I_2 to form some other compound so making the iodine unavailable to thyroid cells(6). The same ratio of activities among the thyroid fractions from both groups of rats suggests that Surital does not interfere with thyroxine synthesis in the cells. Iodine has been shown to react with thioureas and thiouracil derivatives(7), the reaction being not always stoichiometric. This suggests that iodine may react with the thiobarbiturate forming a complex substance from which the iodine cannot be assimilated by the thyroid gland. The action of Surital probably follows the mechanism of action of other antithyroid drugs(6). This study suggests that clinical evaluation of thyroid status via I^{131} uptake should exclude previous treatment with thiobarbiturate anesthetics.

Summary. 1. Surital markedly reduces the incorporation of I^{131} into rat thyroid. 2. The proportion of recoverable I^{131} in the inorganic and organically bound fractions of thyroid alkaline hydrolysate is essentially the same for control and Surital treated rats.

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Incorporation of C^{14}-Carboxyl-Labeled Leucine by Antibody Protein.* (20575)

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An important question in the physiological behavior of proteins is whether proteins can exchange amino acids without losing their original identity. Antibody protein offers an excellent tool for this problem since such proteins can be introduced into the circulation of an animal and removed after suitable time and specifically recovered by precipitation with a homologous antigen. Heidelberger *et al.*(1), carried out an investigation of this type by feeding N^{15} labeled glycine to rabbits which had been given an injection of antipneumococcus rabbit serum. Although subsequent analysis of their passively transferred antibody precipitates gave heavy nitrogen values which were slightly higher than experimental error, they concluded *a priori* that this was the result of non-specific absorption of other serum constituents which had taken up considerable amounts of the labeled glycine. When C^{14} became available, Kooyman and Campbell(2) reinvestigated this problem by injecting C^{14} labeled leucine into rabbits which were also given injections of several different antibodies. Their results indicated that passively transferred homologous antibody acquired considerable radioactivity after several days. However, as a result of some subsequent investigations, the question arose as to whether this activity might not be the result of absorption of non-specific serum proteins as Heidelberger *et al.* had thought possible. In view of this possibility the following investigation was carried out to repeat the previous studies with leucine but to study the problem of contamination more carefully.

Materials and methods. Synthesis of C^{14}-labeled leucine. The synthesis of leucine was

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carried out by the method developed by Dr. Kooyman with only a few minor modifications. This method consisted essentially of reacting a solution of isoamyl magnesium bromide with dry carbon dioxide generated from radioactive barium carbonate.‡ The resulting isocaproic acid was brominated, treated with liquid ammonia and the leucine obtained was recrystallized as described by Marvel(3). The details of this synthesis are hardly necessary at this time since Borsook *et al.*(4) have described a superior method using the Strecker synthesis.

Determination of radioactivity. Protein samples were dried with alcohol and ether and deposited as a fine powder on counting planchets. Complete drying was necessary in order to obtain uniform surfaces on the samples. All the preparations gave similar self-absorption curves. Radioactivity determinations were made with a Geiger-Mueller counter using a Tracerlab 64 scaler. The Geiger tube had an end window thickness of 1.4 mg per cm^2. The final values were corrected to counts per minute per 10 mg of sample making the necessary correction for self-absorption.

Experimental. The first experiment was made on an 8 lb rabbit (rabbit 63) which had been receiving immunizing injections of bovine globulin p-azophenylarsonate (RBG). The last immunizing injection was given on the day that the experiment started. Intraabdominal injections each consisting of 30 ml of a 1.0% solution of the labeled leucine were then given to this rabbit on 3 consecutive days (day 0, 1 and 2). The leucine solution was sterilized by filtration and showed an activity of about 10^5 counts per ml per minute in the counter used. On day 3 the rabbit received 2 intravenous injections of a rabbit antiovalbumin globulin solution spaced about 6 hours apart. This amounted to a total of 27 ml of solution containing 33 mg of protein per ml and approximately 6 mg of precipitable antibody per ml. On the next day (day 4) the animal received 11 ml of horse anti-SSS I and II refined serum (Lederle Labs., Inc.) which contained 49 mg of protein per ml and 12.5 mg of precipitable antibody to type I pneumococcus polysaccharide and 17.0 mg of precipitable antibody to type II pneumococcus polysaccharide per ml. On day 5, 70 ml of blood were taken from the rabbit's ear and this was defibrinated by shaking with glass beads. The rabbit then received 35 ml of sterile saline. On day 7 60 ml of blood were taken from the ear and defibrinated. The defibrinated blood samples were centrifuged and the serum was removed. Small portions of the serum samples were titrated with bovine globulin p-azophenylarsonate to determine the optimum ratio. Using this ratio the active antibody was precipitated from 20 ml portions of the sera. The samples were allowed to remain 48 hours in the refrigerator and the precipitate was then centrifuged down, washed 3 times with cold saline, and prepared for counting. Background was determined before and after each sample was counted. The radioactivities measured with the nine-tenths statistical error are given in Table I. As is shown the RBG antibody precipitates contained appreciable amounts of radioactivity.

TABLE I. Radioactivity of Antibody Proteins from Rabbit 63, Immunized against Bovine Globulin p-Azophenylarsonate and Injected with C^{14}-Labeled Leucine.

Bleed	Wt, mg	Counts/min.	Counts/min. /10 mg
	Active antibody—anti-RBG precipitate		
1	4.3	35.7 ± 2.5	81
2	10.8	58.3 ± 2.5	68
3	18.6	36.8 ± 2.0	32
	Passive homologous antibody—antiovalbumin precipitate		
1	2.7	.5 ± 1.1	
	Repeat	1.6 ± 1.8	
	2.7	.06 ± 1.0	
	Repeat	1.25 ± .8	
2	2.4	−.10 ± 1.0	
3	6.5	.03 ± .8	
	Passive horse antibody—anti-SI SII precipitate		
1	9.4	8.0 ± .4	10
2	2.4	2.0 ± .4	8

Errors given are all 9/10 statistical error.

In the earlier work the question was raised as to the effect of complement and perhaps other non-antibody protein being bound by the precipitates. The presence of radioac-

‡ Obtained from the Oak Ridge National Laboratory operated by Union Carbide and Carbon Corp.

tive material would have little effect upon the specific activity of the active antibody precipitate but could have an effect on precipitates of low specific radioactivity. Assuring the removal of a material as complex as complement would be very difficult, for although complement activity is destroyed by heating, the inactive form might still be absorbed. However, supernatants from the active antibody precipitations which should have little complement activity, if any, were heated at 56°C for half an hour. They were then tested in the standard manner for complement activity(5) and found to be negative. This still did not completely assure the removal of any material which might be carried down with the precipitates in a more or less non-specific manner but it at least made it rather unlikely. The solutions were recentrifuged and titrations were run with ovalbumin to determine the optimal ratios for precipitating the passive rabbit antibody and the precipitations and counting were carried out as for the active antibody. In a similar manner the horse antibodies were precipitated with a mixture of the two polysaccharides in the optimum proportions and counted. The results are also shown in Table I. It was found that the passive homologous antibody contained little or no activity but the horse antibody precipitates contained some activity. This of course is believed to be due to the absorption of other serum components.

These results were contrary to the earlier work of Kooyman and Campbell and it was decided to repeat the experiment with the same animal. The rabbit received a few more injections of the immunizing antigen and then on day 24 it received an intraperitoneal injection of 30 ml of the 1% tagged leucine solution. Over the next 2 days it received 4 intravenous injections of 10 ml of a rabbit antiovalbumin serum which contained about 4 mg of precipitable antibody per ml. On the next (day 27) 55 ml of blood were taken from the animal and this was treated in the same manner as were the previous samples. The results are given in Table I and again show that the transferred rabbit antibody contained no radioactivity.

For a further check on the above results the experiment was repeated with a different animal and different antigen-antibody systems. A healthy female rabbit (Rabbit 361) weighing about 7 lb received a long series of injections of crystalline bovine serum albumin (BSA) solution and had a high titer of anti-bovine serum albumin antibodies. On the 3 days following the last immunizing injection (day 0, 1 and 2) it received 27, 24 and 20 ml of a 1% solution of the labeled leucine. On the next 2 days (day 3 and 4) it received 4 10 ml intravenous injections of a solution of rabbit anti-bovine globulin p-azophenylarsonate serum. This material gave 10.9 mg of precipitate per ml when titrated with bovine globulin p-azophenylarsonate. The rabbit was then bled on days 5, 6, 13, 20 and 27 taking 40, 65, 40, 30 and 30 ml of blood on the respective days. The bleedings were all from the ear and after each bleeding the animal received an intraperitoneal injection of sterile saline. Between the third and fourth bleedings the immunizing injections with bovine serum albumin were again continued. The serum from the bleedings 1 to 5 gave 3.8, 1.8, 2.1, 1.6 and 0.0 mg of precipitate per ml when titrated with BSA and 1.8, 1.0, 0.0, 0.0, 0.0 mg of precipitate per ml when titrated with RBG. The samples were treated in the same

TABLE II. Radioactivity of Antibody Proteins from Rabbit 361, Immunized against Bovine Serum Albumin and Injected with C^{14}-Labeled Leucine.

Bleed	Wt, mg	Counts/min.	Counts/min. /10 mg
	Active antibody—anti-bovine serum albumin precipitate		
1	9.5	29.0 ± 1.4	36
2	10.8	34.5 ± 1.4	40
3	6.7	13.3 ± 1.0	21
4	1.1	1.8 ± .4	16 ± 5
5	No antibody		
	Passive antibody—anti-RBG precipitate		
1	18.0	2.8 ± 1.5 2.2 ± 1.0	2
2	9.4	3.8 ± 1.0	5
3, 4, 5	No antibody		
	Recounts		
1	10.8	1.4 ± 1.0 2.2 ± .6	2
2	6.2	.9 ± 1.2	1.5 ± 2
	Repeated with fresh material		
2	9.6	2.4 ± 1.1	3

Errors given are all 9/10 statistical error.

manner as before, checking for complement activity after the removal of the active antibody. In all cases the active antibody was precipitated first. The amount of radioactivity in each precipitate is given in Table II. It is to be noted that the passive antibody in this case did show some radioactivity although not as much as was found in the earlier work. To determine if this radioactivity was merely the result of absorption the passive antibody precipitates were resuspended in saline and again washed by the procedure outlined. The material from the second bleeding showed a real decrease in the amount of radioactivity. A different sample of the passive antibody material from the second bleeding was washed in a more thorough fashion than the other sample and it was found to contain an intermediate amount of radioactivity. The data are shown in Table II.

It seemed of interest to compare the amount of radioactivity in the antigen-antibody precipitates with that present in the other blood components that were available. The fibrin from each bleeding was washed, dried, and pulvarized and then rewashed and samples for counting were prepared in the same way as the antigen-antibody precipitates. The fibrin activities are given in Fig. 1 and 2. Samples of the erythrocytes from each bleeding were washed repeatedly with saline and were laked with distilled water. Ninety-five per cent ethyl alcohol was added to the clear solutions and the precipitates were centrifuged

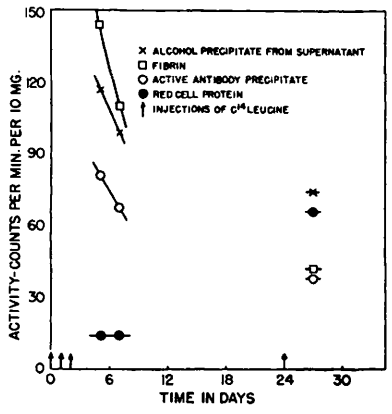

FIG. 1. Radioactivity of various blood proteins in rabbit 63—immunized against bovine globulin p-azophenylarsonate and inj. with C^{14}-labeled leucine.

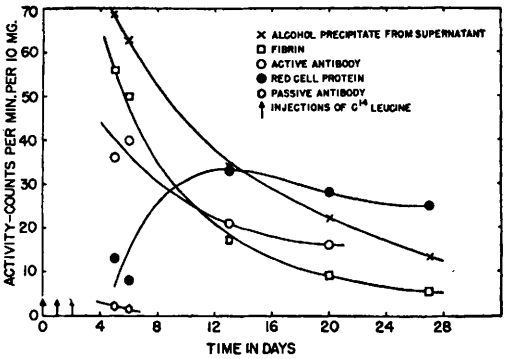

FIG. 2. Radioactivity of various blood proteins in rabbit 361—immunized against bovine albumin and inj. with C^{14}-labeled leucine.

out. The precipitates were treated in the same manner as the other materials and assayed for radioactivity. The amounts of radioactivity found are given in Fig. 1 and 2. The supernatants from the antibody precipitations were treated with alcohol and the resulting precipitates were likewise treated, mounted, and assayed for radioactivity. The amounts of radioactivity found in this material are also given in Fig. 1 and 2. The data for the antigen-antibody precipitates is also plotted in Fig. 1 and 2.

Discussion. The results obtained in these experiments on the incorporation of a C^{14} labeled amino acid by rabbit antibodies are essentially what one would predict from the heavy nitrogen experiments of Heidelberger *et al.*(1) and support their conclusion that the passive antibody does not incorporate the labeled amino acid. As shown above, in some cases the passive antibody material did contain some C^{14} but this could be removed at least to some extent by extended washing of the precipitate and would thus seem to be non-specific. It is to be noted that the passive horse antibody was even more radioactive than the passive rabbit antibody, again indicating that the effect is non-specific. In general great care must be exercised in evaluating the meaning of radioactivity in precipitates such as obtained in these experiments. A great deal of the radioactivity may be non-specific(6). This may or may not account for the earlier results. In some of the earlier experiments the passive antibody was precipitated before precipitating the active antibody and one might expect more

non-specific material to be brought down in the first of a series of antigen-antibody precipitations.

It is possible to estimate the half life of the different serum constituents from curves similar to those of Fig. 1 and 2 if it is assumed that the radioactivity is not part of a molecule that is reutilized to make more of the same material. However, as large samples of blood were taken at each bleeding the data are not too significant in this regard. It is interesting to note that the red cells are still incorporating activity when the activity of the other serum constituents is decreasing rapidly.

These results confirm those of Schoenheimer and Heidelberger. The normal constituents of a rabbit's serum incorporate radioactivity given in the form of an amino acid at a very rapid rate. Passively transferred antibodies either do not incorporate this radioactivity to any marked extent or else do so only upon the loss of their specific antibody characteristics.

Summary. C^{14}-labeled leucine has been administered to immune rabbits. It has been found that while the normal serum components incorporate the C^{14} activity passively transferred antibody does not.

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Studies of 11-Alpha Hydrocortisone* in the Chick Embryo and on Skin Lesions in Man.† (20576)

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Cortisone acetate has a marked, characteristic effect in modifying and inhibiting growth of the developing chick embryo(1). Because of the specificity of this effect, the chick embryo has been used to examine related steroids for "cortisone activity"(2,3), and affords a useful and simple method for testing compounds for adrenocortical activity. Compound F (17-hydroxycorticosterone) acetate was found to be 50 to 100 times more active than cortisone acetate in the chick embryo and the newly-hatched chick(4); this marked difference was not noted in comparative studies on mammals.

The growth-inhibiting dose of hydrocorti-

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