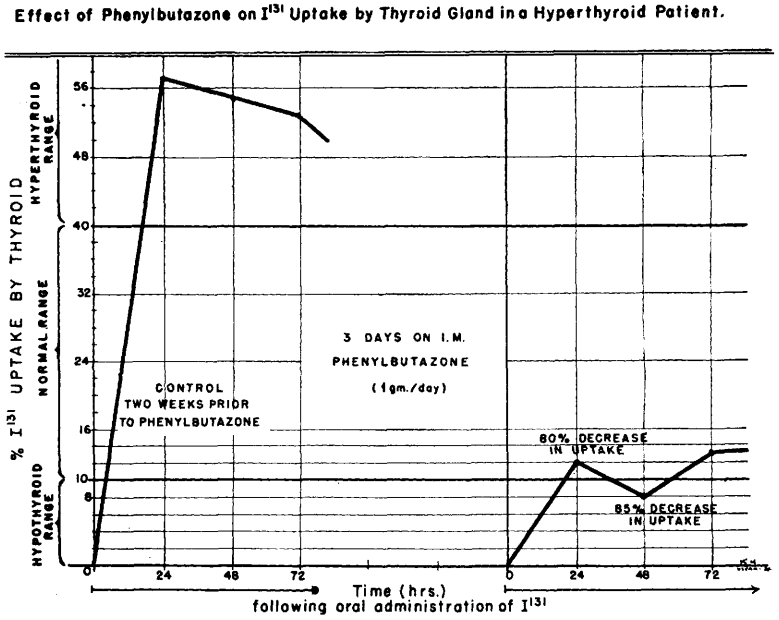

FIG. 2.

thyroid) butazolidin[R] interfered with uptake of I^{131} in the thyroid gland. The extent of this action is now being observed through a series of I^{131} uptakes during the continuous prolonged oral administration of butazolidin[R].

At the present time it may be stated that no instance of clinical hypothyroidism has been observed in our group of gout and arthritic patients, some of whom have been treated with the drug for as long as 12 months.

The mode of action of this compound on the thyroid was unknown at the time of these observations; however, possible mechanisms are suggested in another publication.†

Summary. The intramuscular injection of butazolidin[R] in a daily dose of 1.0 g for 2-3 days reduces I^{131} uptake by the thyroid gland.

† Effect of Butazolidin[R] upon the Fate of I^{131} in the Rat-I, Scott, Kenneth G., Frerichs, John B., and Reilly, William A.

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Effect of Pooling of Liver Slices upon Protein Synthesis in Guinea Pigs. (20052)

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Assuming the radiation emitted by S^{35}-methionine incorporated *in vitro* into liver protein to be x counts/min/unit in one animal, and y counts/min/unit in another, if the 2 livers were pooled, in equal units, a value of x + y/2 counts/min/unit would be expected. In the course of work involving guinea pigs, it was noted that pooled liver slices did not incorporate S^{35}-methionine at the rate expected from the samples singly; hence the following study was made. As will be shown, pooled values were consistently lower than theoretical values.

Materials and methods. Female guinea pigs

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of mixed breeds and colors, weighing 350-600 g, were used. Water and standard rabbit ration (Red Chain) were supplied *ad lib.*, and fresh vegetables were given 5 days per week. The guinea pigs were killed by a blow to the head, and the livers immediately removed and placed on ice. Slices were made free-hand with razor blades and placed in cold buffer of the following composition per 100 ml:

70.2	ml .154 M	NaCl
1.4	.11	CaCl
1.4	.154	KCl
27		M/10 phosphate pH 7.25
200	mg glucose	

After the liver was sliced, the buffer was absorbed with gauze, and the slices weighed. Ten g of slices and 10 ml of buffer were placed in a 125 ml Erlenmeyer flask. S^{35} labeled L-methionine† was added, so that a concentration of about one microcurie/ml was present. The amount of methionine varied from 0.35 to 3.0 mg, but was constant for a given experiment. The time from death of the animal to placing the slices on the bath was about 90 minutes in all cases.

A typical experiment. Two female guinea pigs, A and B, weighing 405 and 384 g respectively, were killed and the livers sliced. Flask 1 contained 10 g of liver from A and 9.65 ml of buffer. Flask 2 contained 10 g of liver from B and 9.65 ml of buffer. Flask 3 contained 5 g of liver from A, 5 g from B, and 9.65 ml of buffer. To each of the 3 flasks was added 0.35 ml of solution containing 19.8 μc S^{35} and 1.6 mg of L-methionine. The flasks were placed in a Warburg bath at 37°C, allowed to equilibrate, and stoppered. They were shaken for 2 hours and then opened for 5 minutes to permit renewal of air. After a total of 4 hours, the flasks were removed from the bath and the contents immediately homogenized in a cold room at 3°C. The liver protein was precipitated by the addition of an equal volume of 10% trichloroacetic acid. The time from removal from the bath until the protein was precipitated was 15 minutes. The precipitates were washed three times with 0.05 M non-radioactive DL-methionine, and once with water. The residues were spread on porcelain Petri dishes and dried overnight under a 60-watt lamp. The dried material was ground in a mortar and 300 to 600 mg were placed in planchets of 4.49 cm^2 area. The surfaces were tamped level. Specimens were set up in duplicate. Each sample was counted twice in a Nucleometer,‡ at infinite thickness for a total of 12,800 counts each. Infinite thickness was determined to be <200 mg/4.49 cm^2. All raw counts were >10 x background. The mean variation in counts between the 41 pairs of duplicates set up in separate planchets was 3.4%. To determine *adsorption of S^{35}-methionine,* the procedure was carried out as above at zero time after mixing. The recorded experimental figures have been corrected for this adsorption. All counts were normalized to the basis of 1 μc S^{35}/ml of the flask contents. All counts are expressed as counts per minute 4.49 cm^2.

Results. Adsorbed methionine. In Exp. 1, performed at zero time, the first guinea

† Purchased from Abbott Laboratories, N. Chicago, Ill.

TABLE I. Error of the Method.

Exp. No.	Guinea pig No.	CPM/ 4.49 cm^2	Variation, %
2	1	3840	7.3
	Duplicate	3560	
3	2	3590	8.2
	Duplicate	3910	
4	3	4560	4.6
	Duplicate	4570	
	Triplicate	4360	

Identical flasks were set up for 3 control animals.

TABLE II. Effect of Pooling on Guinea Pig Liver Protein Synthesis.

Exp. No.	A	B	½(A+B)	C	$\frac{C}{\frac{1}{2}(A+B)} \times 100$
5	6250	4940	5600	3260	58
6	2690	3860	3270	1990	61
7	4850	3390	4130	2560	62
8	8230	3970	6100	3870	63
9	3350	3400	3380	2180	64
10	6370	4280	5320	3440	64
11	3730	3450	3570	2380	66

Values are counts/minutes/4.49 cm^2.
A = value from first animal.
B = " " second " .
C = " " equal parts of first and second animals.

‡ Internal gas flow radiation counting meter, manufactured by Radiation Counter Laboratories, Skokie, Ill.

TABLE III. Effect of Heat and Supernate upon Pooling Effects.

Exp. No.	Ingredients	A	B	½(A+B)	C	$\frac{C}{½(A+B)} \times 100$
12	Normal slices (A)+ heated slices (B)	9120	0	4560	5610	123
13	Normal slices (A)+ normal + supernate (C)	7090			11380	*160
14	Supernate added to pooled sample (C)	4190	11580	7890	5150	65

Values are counts/minutes/4.49 cm².
* In this case, the expected value for C is the same as A.

pig adsorbed 200 CPM, the second 230 CPM, and the pooled sample 213 CPM. The round figure of 200 CPM was used as the adsorption correction.

Error of method. The reproducibility of the method is shown in Table I. In these 3 experiments, duplicates or triplicates were set up and run as described. The overall error of the method appears to be around 8%.

Effect of pooling. Table II represents the results in pooled livers. Although there was a 3-fold variation between the lowest and highest uptake, the percent of the theoretical pooled uptake was amazingly constant, being between 58 and 66% of the theoretical 100%. The mean uptake of the unpooled specimens was 4480 CPM. For convenience, the experiments are arranged with percentages in ascending order, although this was not the experimental sequence.

Effect of heating and supernate. Table III demonstrates the effect of heat and supernate on S^{35} uptake. In Exp. 12, one liver was heated to 80°C for 60 minutes on a water bath, and the experiment then carried out as usual. A moderate stimulation was found. In experiment 13, one liver was sliced and placed on the Warburg without S^{35} methionine. After shaking 4 hours at 37°C, the slices were spun down and the supernate frozen. The following morning another guinea pig liver was sliced and one flask set up as usual. A duplicate was set up with 7 ml of buffer replaced by 7 ml of supernate prepared the preceding day. This second flask showed a marked increase over the flask with buffer alone. In Exp. 14, flasks A and B were set up as usual, but to the pooled slices in flask C was added an equal volume of supernate from a third guinea pig, prepared as described for Exp. 13. A depression to 65%, the same value found without added supernate, was found.

Discussion. No reports could be found on changes of *in vitro* metabolism incurred by the pooling of tissues. Rutman, Dempster and Tarver(1) reported variations in the uptake of S^{35}-methionine by rat liver slices to be dependent upon the strain used. Canzarelli, Rapport and Guild(2) report that the uptake of C^{14}-alanine into rat liver slices can be decreased to 17% of normal by carbohydrate deprivation for 24 hours. A combination of these factors probably accounts for the range of uptake in our animals, although they were feeding *ad lib.* However, this has no obvious relevance to the "pooling effect." Since the period of contact was only 4 hours, antibodies could scarcely be expected to have formed. Furthermore, even a previously immunized animal produces little antibody in the liver(3). Hence, some genetic mechanism other than antigenic differences must be acting. The guinea pigs were not inbred, and all color combinations were used, but no differences in depression were found.

Although the substance, or substances, responsible for this depression must go from one set of cells to the other, Exp. 12, using prepared supernate, did not confirm this concept. A stimulation, rather than a depression, was found. Such an increase could be due to the addition of substrate in the supernate, since net hydrolysis occurs in all slice experiments, and after 4 hours incubation the amino nitrogen is always increased. Exp. 13, using heated tissue, would also release substrates, and it,

too was stimulatory. Despite these stimulatory effects, Exp. 14 demonstrated that the addition of such substrates will not alter a depressing effect between two sets of different but viable cells. Much more work is needed to elucidate the mechanisms involved in these observations.

Summary. 1. The uptake of S^{35}-L-methionine into the protein of pooled and non-pooled liver slices in guinea pigs has been studied. 2. Defining the mean of liver uptakes from 2 animals as 100%, when the two guinea pig livers were pooled, an uptake of 58 to 66% was found. 3. No evidence for a diffusible inhibitor could be demonstrated.

1. Rutman, R., Dempster, E., and Tarver, H., *J. Biol. Chem.*, 1949, v177, 491.

2. Canzanelli, A., Rapport, D., and Guild, R., *J. Biol. Chem.*, 1950, v183, 291.

3. Ranney, H. M., and London, I. M., *Fed. Proc.*, 1951, v10, 652.

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Changes in Eosinophil Count of Mice with Venisections* Repeated at Intervals of Several Days.† (20053)

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In the course of studies on characteristic eosinophil levels(1) it was noted that the mean eosinophil count of groups of inbred mice obtained at four-day intervals showed marked differences from one sampling time to the other. Such results were obtained despite the observance of certain precautions to control the physiological eosinophil rhythm(2). These included 1) not only the procedure of sampling at the same times of day, but 2) the equally relevant standardization of experimental animals by means of a regimen of single housing for at least nine days in an air conditioned room at the temperature of 78 ± 2°F with standardized lighting (light from 06:00 to 18:00 and darkness from 18:00 to 06:00)‡ and *ad libitum* feeding.

The methods of handling and counting have been described(1). The counts were made at the times indicated in the tables. The periods chosen are during the rising phase of the usual daily rhythm. It is noted that 2 counts were obtained on each of the days of observation at an interval of 4 hours. The first of the 2 counts for each animal and for each day of observation is presented.

A summary of the data on eosinophil counts obtained under these circumstances is presented in Table I. It is apparent that the mean count obtained for the third venisection is lower than that obtained for the first venisection for each of the 10 stocks of mice investigated.§ It appeared undesirable to accept these observations as representative of a more general phenomenon in mice, since the data were obtained from very homogeneous populations of animals. It does not follow *a priori* that results valid for particular stocks and circumstances would necessarily be valid for other biological material, even though closely related. Yet it appears that they are valid.

* Although the term venisection is employed for linguistic convenience it is recognized that the samples of blood obtained from a small incision in the mouse's tail were either venous or arterial, or mixed. For the purposes of this investigation it is believed that the distinction between venous and arterial blood is not significant.

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‡ Standardized environmental circumstances, especially controlled lighting, appear to be necessary to assure constancy of the temporal placement of physiological eosinopenia within the 24-hour period (3).

§ These mice have been described(1).