biotics on growth are exerted independently of any effect on thyroid function. However, it does not preclude the possibility that marked changes in thyroid activity can alter normal nutritional requirements of the body. Thus hypothyroidism generally decreases body growth and is believed to reduce dietary requirements, while severe hyperthyroidism also reduces body growth but is believed to increase nutritional requirements(12). If one considers normal body growth as a criterion of adequate dietary intake, then the favorable effects of vit. B_{12} or antibiotics can be considered as indicative of increased nutritional needs under either thyroid condition.

Summary. 1. White Leghorn male and Rhode Island Red chicks of both sexes were fed a vit. B_{12}-deficient ration which was supplemented with 0.1% thiouracil and/or 0.5 to 4.0 μg % vit. B_{12} or 2 mg procaine penicillin G per lb of food. After a preliminary depletion period of one and 2 weeks, respectively, on the vit. B_{12}-deficient diet, the chicks were fed the above supplements for an additional 4 or 3 week period. 2. Thiouracil markedly reduced body weight gains and food intake in both breeds of chicks, and increased the grams of food required for each gram of gain in body weight. Vit. B_{12} and penicillin partially or completely counteracted this growth depression and increased appetite. The larger doses of vit. B_{12} were more effective in these respects than the smaller doses. 3. The large increase in thyroid weight and severe inhibition of comb growth induced by thiouracil were not influenced by the vitamin or antibiotic. 4. In general, these results corroborate previous data on the effects of vit. B_{12} and antibiotics on rats fed thiouracil or following parathyroidectomy.

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Volume of Distribution of Radiosulfate as a Measure of the Extracellular Fluid. (19382)

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Virtual equilibrium following the injection of certain tracer ions, such as Na^+ and Cl^-, requires several hours or days, presumably because exchange rates with some of the intracellular unlabelled ion are very slow(1,2). On the other hand, substances such as mannitol, which are largely excluded from body cells, may attain equilibrium throughout the extracellular fluid within 50 minutes, even though slow cell penetration occurs thereafter(3). Sulfate, as a consequence of its rapid capillary exchange rate(4), might be expected to attain equilibrium throughout the extracellular fluid even more rapidly. Studies with unlabelled sulfate indicated that this is the case(5).

Studies were undertaken of the distribution and excretion of radiosulfate in normal human subjects. S-35 has a convenient half-life of 87 days and can be administered to man without hazard.* A dose of 100 μc† was injected in-

travenously from calibrated 5 cc syringes during the course of one minute. Samples of serum or plasma drawn at intervals after the end of injection and an aliquot of the injected material appropriately diluted were enriched with sodium sulfate to minimize errors due to changes in self-absorption. Serum proteins were precipitated with trichloracetic acid. Sulfate was precipitated as the benzidine salt and collected on filter papers, which were permanently mounted on aluminum discs(6). Urine samples, obtained without the use of catheters, but with sufficiently great urine flow in most cases to minimize dead space errors, were analyzed similarly except that the amount of sulfate precipitated was determined by weighing each sample. Self-absorption corrections relative to the weight of the plasma samples were applied from an empirically determined curve. Radioactivity was measured by mica-window Geiger counter tube. Duplicates differed by an average of 0.7%.

Fig. 1 shows a composite concentration-time curve of plasma activity for 7 normal subjects. Values during the first 13 minutes

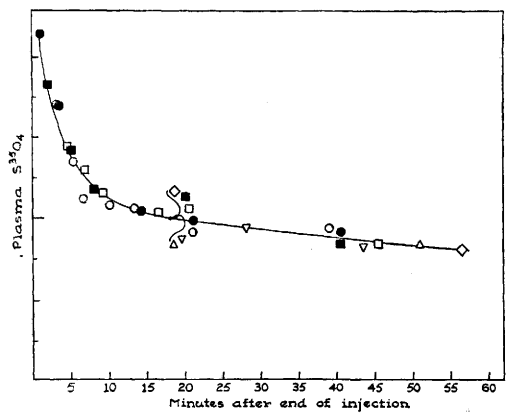

FIG. 1. A composite concentration-time curve of plasma activity for 7 subjects. For each subject (represented by a single type of symbol) curves were adjusted to the same 18 min value by appropriate factors. A curve of best fit for the entire group was drawn visually.

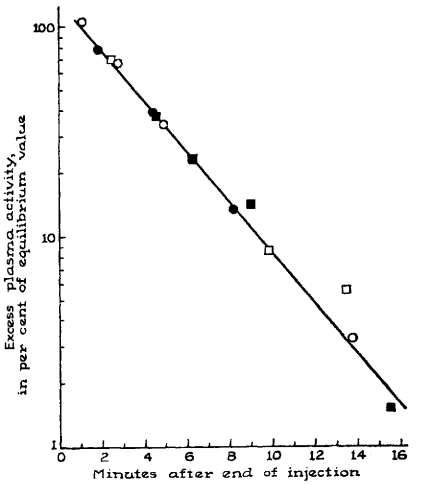

FIG. 2. Equilibration of radioactive sulfate. Excess plasma activity over the later slow rate of disappearance is plotted against time on a semi-logarithmic scale for four subjects. The line was drawn visually.

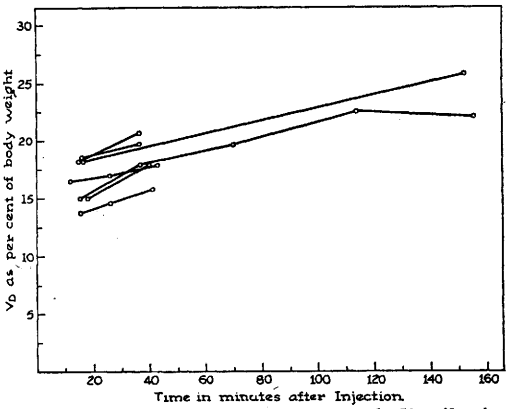

FIG. 3. The increase in volume of distribution (V_D) of radioactive sulfate with time in seven normal subjects. Volumes are expressed as % of body wt.

were obtained from femoral arterial samples in order to reduce fluctuations due to intravascular mixing, in 4 of the 7 subjects.

The curves for these 4 subjects were analyzed into 2 additive rates. This was done by extrapolating the later, straight part of the curve back to zero time on a semi-logarithmic plot and tabulating the differences between this line and the obtained values at each point. This difference is plotted semi-logarithmically against time in Fig. 2. For each subject a straight line with a decay constant of approximately 0.28 is obtained. The difference is plotted as per cent of the 18 minute value for

* 100 microcuries, if wholly retained in the body of a 70 kg man and eventually decayed there, would produce only 0.6 roengten equivalents physical.

† The S-35 labelled sulfate was obtained from the Oak Ridge National Laboratories on authorization by the Atomic Energy Commission.

TABLE I. Volume of Distribution at 18 Min of Radioactive Sulfate in 10 Normal Subjects.

Subject	Height, cm	Sex	Wt, kg	Vol of distribution, l	Body wt, %
H	173	♂	75	14	18.7
W	183	♂	78.8	13.4	17
MW	182	♂	70	10.6	15.1
B	175	♂	82.5	13.8	16.8
J	185	♂	84.6	13.8	16.3
N	170	♀	54.7	10	18.4
BL	164	♀	48.4	8.2	16.9
LS	162	♀	65.2	8.4	12.9
S	177	♂	79.6	12.2	15.3
JM	180	♂	43.3	6.8	15.7
				Avg	16.3

convenience in order to combine the results for all 4 subjects.

From 15-20 minutes on, the rate of fall of the plasma concentration is much slower (decay constants .003-.007).

In order to determine the extent to which urinary loss accounted for this later slow fall in the plasma concentration, the volume of distribution was measured at intervals following injection. It was calculated as the amount injected minus the amount excreted divided by the interstitial fluid concentration.‡ Eighteen minutes was selected as the first interval since the early rapid rate appeared to be completed at this time. The results are shown in Fig. 3. A progressive increase in the volume of distribution with time is noted in all subjects.

These results were interpreted to mean that radiosulfate becomes distributed throughout a certain compartment within 15-20 minutes, and slowly penetrates beyond it thereafter.

Two alternative hypotheses were considered: first, that this compartment represents only the more accessible portions of the extracellular fluid, and that penetration into the remainder of the extracellular fluid proceeds at a much slower rate; and secondly, that this compartment corresponds to the anatomic extracellular space and further penetration represents cellular uptake. A slow rate of uptake by bone and cartilage has been observed(7).

‡ The plasma water concentration was divided by 0.90 to obtain interstitial fluid concentration. This is a reasonable approximation for the Gibbs-Donnan factor for divalent ions. No experimentally determined factor for sulfate was found in the literature.

In order to attempt to answer this question the magnitude of the 18 minute space was determined in normal subjects. The results are shown in Table I. It should be noted that omission of corrections for plasma water and Donnan effect would increase each space 12%, changing the average value to 19.5% of body weight. The corrected mean of 16.3% body weight corresponds closely to the figure of 16% for ECF given in a recent review of compartment measurements by Levitt and Gaudino(8).

The constancy of this compartment was determined by serial measurements at intervals of several days in 6 individuals. In 9 out of 10 instances, the volumes checked within 0.3 liter.

These results tend to support the second hypothesis—that sulfate becomes distributed throughout the extracellular space within 15-20 minutes. It also seems improbable on *a priori* grounds that penetration into the interstitial fluid should proceed at 2 so vastly different rates, but this possibility cannot be excluded at present. Sheatz and Wilde reported rapid disappearance of radiosulfate in rats, but found volumes of distribution 34% of body weight 30 minutes after injection(4). The explanation of the discrepancy between this value for rats and the value for man reported here is not evident.

Summary. Radiosulfate becomes distributed within 15 to 20 minutes in a reproducible space which is 16% of the body weight in man. It is suggested that this space may correspond to the extracellular fluid.

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Bromsulfalein Retention in Experimental Obstructive Jaundice. (19383)

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(Introduced by P. György.)

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In a previous study, prolonged retention of bromsulfalein (BSP) was observed in patients with regurgitation jaundice(1). The present experiments were performed to study this phenomenon in the laboratory animal. The work of Snell, Greene, and Rowntree(2), and Cantarow and Wirts(3) suggested that phenoltetrachlorphthalein in the dog and bromsulfalein (phenoltetrabromphthalein) in the cat were cleared from the plasma rather rapidly despite complete biliary obstruction. These observations have been extended in the present study of BSP retention in dogs and rats with common bile duct ligation.

Material and methods. Six mongrel dogs were anesthetized with intravenous pentobarbital and the common bile duct of each was sectioned between 2 ligatures. Following complete recovery, BSP (5 mg per kilo) was injected intravenously. Tests were done at weekly intervals in each animal for an average of 3 weeks. Serum was analyzed for dye before each injection, and at ½, 24, 48 and 72 hours after the injection. The same operation was performed in 13 Sprague-Dawley rats under ether anesthesia. After recovery, 5 mg of BSP per 100 grams of body weight were injected intravenously or intraperitoneally. Serum obtained by cardiac puncture was analyzed at the same intervals as for the dogs. One test only was done in each rat. The method of Gaebler(4) using the Evelyn photoelectric colorimeter was employed in determining serum concentration of dye. Abnormal retention was considered present if the concentration exceeded 0.5 mg % in dogs(5) (equivalent to 5% retention), and 1.0 mg % in rats(6).

TABLE I. Duration of BSP Retention in Experimental Obstructive Jaundice.

	Dog*	Rat*
< 24 hr	9	0
24-48 hr	8	9†
48-72 hr	2	4‡

* No. of tests performed.
† Dye inj. intrap.
‡ " " intrav.

TABLE II. Average Daily Urinary Excretion of BSP in Experimental Obstructive Jaundice.*

Day	Dog	Rat
1	42	22
2	2	7
3	0	1
4	0	0
Total	44	30

* % of amount inj.

Results. In Table I, it will be seen that in dogs with complete biliary obstruction, with serum bilirubin values ranging from 8-12 mg %, BSP was removed from the serum within 72 hours. A similar rapid clearance was also observed in rats with common bile duct obstruction. The serum bilirubin in these animals was 5-6 mg %.

Analyses were carried out to determine how much of the retained dye was excreted. An