unknown. With the collaboration of Dr. F. Kull and Miss M. Grimm of our laboratories, we have examined their influence upon various enzyme systems and found that their antituberculosis action is not based upon an antiphenoloxidase activity so pronounced with thiourea and certain monosubstituted thioureas nor due to an effect upon cytochrome C. A very high *in vitro* activity against different strains of torula, which contain as do the mycobacteria, a high percentage of lipids, may indicate a possible pathway for the tuberculostatic activity of these thioureas.

Summary. 1. Over 350 thioureas have been prepared in our laboratories and tested for antitubercular activity. 2. A number of disubstituted thioureas have demonstrated considerable protective and therapeutic activities in tuberculous mice and guinea pigs. 3. Little correlation existed between the *in vitro* and *in vivo* tuberculostatic action. 4. Many compounds possessed strong antifungal as well as antimycobacterial properties.

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Determination of Specific Gravity of Intact Animals by Helium Displacement; Comparison with Water Displacement.* (20242)

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Interest in the concept that the body is composed of a lean tissue mass of constant composition upon which is superimposed a variable amount of fat was stimulated by the work of Behnke(1). In normal men(2) and animals(3), the weight of fat-free tissue in the body as a whole or in individual tissues(4) is more closely correlated with the water and electrolyte content than is the weight of the total body or tissue. Since fat is relatively inert metabolically, the lean body mass may also be a more valuable referent for total metabolism than the surface area(5). Since the specific gravity of fat is about 0.92 and the specific gravity of the fat-free body is about 1.100(2), the proportion of body weight which is lean tissue and, conversely, the proportion which is fat, may be calculated from the whole body specific gravity. In man, specific gravity may be determined by underwater weighing, if correction is applied for the residual air in the lungs. This procedure is difficult to perform because it requires considerable training and cooperation on the part of the subject, and it is not feasible in incapacitated individuals. In intact animals this procedure is obviously impractical, and it is necessary to sacrifice and eviscerate animals in order to find their weight under water.

The method herein reported permits rapid determinations of specific gravity in intact, unanesthetized animals. Body volume is measured by gas displacement. Weight (g) divided by volume (cc) gives specific gravity, and no correction for residual air is needed.

Methods. Principle. A measured quantity of helium is added to a chamber containing the animal. After complete mixing of the gas with the air in the chamber and in the animal's lungs, a sample is obtained for analysis. Then, by the dilution principle: Volume of empty chamber $-\frac{\text{Vol. of gas added}}{\text{Final conc. of added gas}} =$ vol. of animal. Helium was selected as the referent

* The opinions expressed herein are those of the authors and should not be construed as necessarily representing the opinion of the naval service.

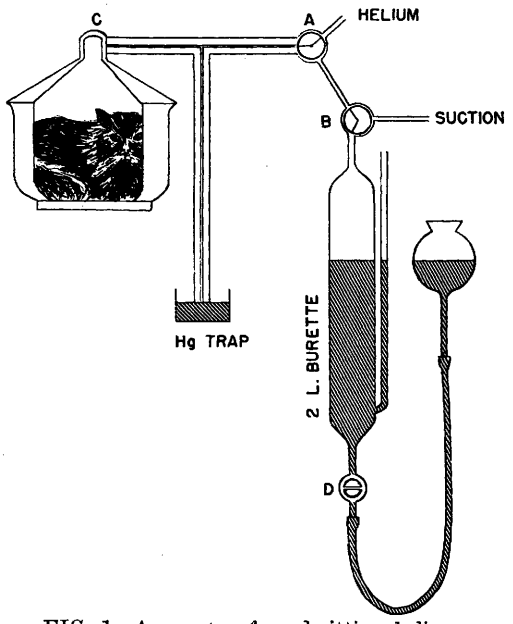

FIG. 1. Apparatus for admitting helium.

gas because it is inert, diffuses rapidly, and is the least soluble of the gases. Instead of enlarging the chamber in order to admit the helium at atmospheric pressure, it proved more convenient to evacuate the chamber partially and then add helium until atmospheric pressure was restored. *Apparatus* (Fig. 1). A 2-liter calibrated gas burette was filled with helium under slight positive pressure through stopcocks A and B. The pressure was reduced to ambient by releasing helium through the mercury trap, and the level of mercury in the burette recorded. (In view of the low solubility of helium in water, the use of mercury instead of water to displace helium was probably an unnecessary precaution.) The animal was placed in the chamber (an ordinary desiccator jar of exactly 6-liter capacity), and a negative pressure of approximately 20 cm Hg (measured by the height of the mercury above the trap) was produced therein by evacuation through C, A and B. Helium was admitted to the chamber from the burette through stopcocks B, A and C, by carefully manipulating stopcocks B and D, in order to maintain the pressure within the burette close to atmospheric. Thus it was possible to prevent temperature change in the burette. When the pressure within the chamber was restored to ambient, as indicated by the fall of the mercury above the trap to the level in the trap, stopcock C was closed. This procedure required less than one minute. The burette was read again and the volume of helium added obtained by difference. After 3-4 minutes (see below) a sample was obtained from the chamber for analysis and the animal was removed. *Experimental animals.* Cats weighing 2 to 3 kg were used. Most of the fur was removed with clippers before the experiment in order to facilitate subsequent underwater weighing of the carcass; air trapped in the fur was otherwise difficult to remove. *Analysis of helium.* Helium was de-

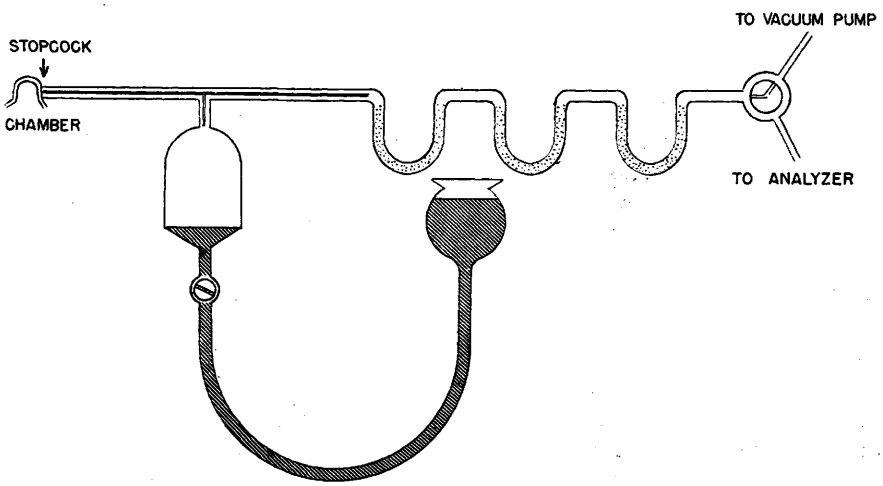

FIG. 2. Gas sampling apparatus.

TABLE I. Comparison of Specific Gravity Determinations by Gas Displacement and Water Displacement in 10 Cats.

Subject	Wt, g	Sp. gr. by He_2 Values	Mean	Sp. gr. by H_2O	Difference
H_2O	4000	.998 .991 .991	.993	.998*	—.005
Cat # 1	2605	1.080 1.070 1.070	1.073	1.050	+.023
2	2605	1.035 1.050 1.034	1.039	1.040	—.001
3	2519	1.023 1.051 1.027	1.033	1.048	—.015
4	2235	1.060 1.056	1.058	1.059	—.001
5	2552	1.083 1.074 1.073	1.077	1.071	+.006
6	2009	1.061 1.076	1.068	1.067	+.001
7	2060	1.087 1.083	1.085	1.077	+.008
8	2755	1.032 1.046	1.039	1.071	—.032
9	2512	1.057 1.067 1.039	1.054	1.051	+.003
10	2074	1.035 1.027	1.031	1.071	—.040
			Mean difference		.013
			Algebraic mean		—.005

* Density of water at room temperature.

termined in a Cambridge Analyzer, which utilizes the thermal conductivity of mixtures of helium and dry air to give per cent helium concentration. The gas sample was taken into an evacuated system (Fig. 2), passed through a drying agent and through the analyzer at a controlled rate of flow. *Determination of specific gravity by water displacement.* After sacrificing the animals and removing their lungs, the weight of the carcass suspended in water was determined. Care was taken to remove all air in the respiratory passages and that trapped in the remaining fur. The specific gravity of the lung-free carcass was calculated as:

$$\text{S.G.} = \frac{\text{Wt in air}}{\text{Wt in air—wt in water}}$$

Results. The results of the specific gravity determinations by helium displacement and by water displacement are shown in Table I. The arithmetic mean of differences between results obtained by the two methods was 1.3%. The validity of this procedure for measuring volume is indicated by the results obtained with a known quantity of water, also shown in Table I.

Discussion. Possible sources of error in gas displacement method. 1. Temperature effects: Since the 2 measurements, volume of gas added and helium concentration, are made at sites where the temperature and pressure are equal to ambient temperature and pressure, heating or cooling within the chamber does not affect the results, even though it alters the pressure. 2. Absorption of helium by the body: The solubility of helium in body water is approximately 0.0087 cc/cc(1), and in body fat approximately 0.0148 cc/cc(1). Complete equilibration of body water and fat with a helium atmosphere requires several hours(1). Consequently, the amount of helium removed from the atmosphere surrounding the animal during the time required for mixing is a negligible proportion of the total helium present, and will not affect the results; simple calculations show that even when complete equilibration is obtained the error is small. 3. Incomplete mixing: During normal respiration, equilibration of a foreign gas with alveolar air is 98% complete within 4 minutes(6). The reduction in pressure and restoration of atmospheric pressure by helium addition ensures that all gas spaces in or about the animal which are in communication with the atmosphere will be penetrated by helium, although equilibration with poorly ventilated recesses such as the paranasal sinuses may be incomplete. Mixing within the chamber was aided by the movements of the animals. That mixing was essentially complete was indicated by the fact that leaving a cat within the chamber for 3, 5 or 7 minutes gave the same result. Gas enclosed in spaces such as the stomach and intestines, will not be penetrated by helium. Consequently the results obtained by both the helium dilution and water weighing methods differ from the true body specific gravity by a small and variable amount.

Evaluation of gas displacement method. The results presented show that the volume of intact animals may be determined with fair

precision and accuracy by utilizing the gas dilution principle presented above. By varying the size of the chamber and the gas burette, it should be possible to perform repeated specific gravity determinations on intact animals of most species by this method. Knowledge of the empirical constants relating specific gravity to body fat and body water content should thus permit these latter quantities to be determined with ease and moderate precision.

Summary. A method of measuring specific gravity, and thereby estimating total body water and fat content in intact animals is presented. The dilution volume of helium, in a closed chamber of known capacity containing the animal, is measured, and subtracted from the volume of the empty chamber. This gives the volume of the animal. Weight/volume gives specific gravity. No correction for residual air is necessary. The results on 10 cats by this method corresponded closely to results obtained by underwater weighing of each carcass.

NOTE—Since this work was completed, an abstract has appeared(7) indicating that a helium dilution procedure has recently been applied to body volume measurement in man.

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Gonadotropins of the Pituitary Gland and Urine of the Adult Human Male. (20243)

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The repair of the gonadal atrophy occurring in hypophysectomized male and female rats provides a specific method for the characterization and assay of pituitary gonadotropins. Considerable information on the gonadotropins of the pituitary gland of the hog and the sheep has been derived by use of this method(1,2). However, only few definitive studies of the nature and amounts of gonadotropins of the pituitary gland of the human have been made, and much of what is known has been inferred from investigations on the gonadotropins of human urine(3-8). The purpose of this paper is to present comparative data on the gonadotropins of both the pituitary gland and the urine of the adult human male.

I. Materials and methods. a) Animals. Male and female Wistar rats (30 to 45 days old) were hypophysectomized by the parapharyngeal route. Postoperatively the animals received a solution containing 5% glucose and 0.9% sodium chloride as their drinking water and were allowed Friskie meal *ad libitum.* Beginning on the sixth postoperative day and extending for 4 days, each test animal received twice daily subcutaneous injections (1 cc) of the material to be tested. Groups of uninjected hypophysectomized and intact animals were included in each experiment. On the tenth postoperative day all the rats were killed by ether asphyxiation. A fairly complete gross and microscopic examination of the endocrine glands and other related organs was made. *b) Pituitary tissue.* The pituitary glands of 3 adult human males were obtained within 3 to 4 hours following sudden death.* None of the 3 had had any obvious endocrinopathy and in no case was there any clinical evidence of impairment of the pituitary-gonad system. The ages of the patients were 38, 55 and 61 years. The anterior lobe of each hypophysis was dissected free from the remainder of the