

taneously daily for 15 days with 200 γ of androsterone.† The other one of each pair received no treatment. Control and experimental animals were sacrificed on the 16th day of the experiment. Mammary glands from both groups were removed and studied as whole mounts. The pituitary glands were removed, weighed, and assayed for their lactogen content by injecting the suspended tissue intradermally over the crop glands of common pigeons. One-sixth of a control pituitary gland was injected (divided into 4 daily doses) over the right crop glands of 2 pigeons and one-sixth over the left crop glands of 2 additional pigeons. An equal amount of experimental pituitary tissue was injected over the left crop glands of the first pair of pigeons and one-sixth over the right crop glands of the second pair of pigeons.

The mean difference of the individual comparisons of the weights of the pituitary glands was not significant. The lactogen assay results revealed that 8 of the pituitaries from the injected rats contained more lactogen than did the glands from control rats, 2 were equal to, and 5 contained less lactogen. The mean difference of the lactogen content of the pituitary glands was not significant. At the time of sacrifice the mean difference of the body weights was significant, the control animals weighing more than the injected animals. The mammary glands of the control and experimental rats were similar in appearance, involutionary changes being observed in both groups of glands.

Conclusions. The daily administration of 200 γ of androsterone for 15 days into sexually mature spayed rats caused no significant change either in pituitary weight or pituitary lactogen content and produced no detectable growth of the mammary glands.

11961 P

Use of Helium for Determination of Pulmonary Capacity.

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Hydrogen has been used for the determination of the pulmonary

† The androsterone used in this study was kindly supplied by Dr. E. Schwenk of the Schering Corporation.

capacity by VanSlyke and Binger,¹ who pointed out the danger of explosion and of poisoning with arsine. This procedure was replaced by oxygen dilution methods, particularly the Christie method,³ which has been in use in this laboratory for the last 10 years. A recent report² has resulted in new interest in hydrogen, because of the ease and rapidity of analysis for this gas by thermal conductivity. A preliminary report is made at this time of a new method employing helium, because it has all the advantages and none of the dangers of the hydrogen method.

A closed spirometer system with a blower is used. Carbon dioxide is absorbed by soda-lime. An important precaution is the addition of a water bottle to replace water vapor lost in the soda-lime. Dead space is measured by the oxygen dilution method. Part of the mixture circulating in the respiratory system is shunted through a thermal conductivity cell* with precautions to assure complete saturation of the gas mixture with water vapor. A meter, which is connected to the cell, is calibrated for helium in air fully saturated with water vapor over a range of 0-14% and also for variation in oxygen concentration of the mixture from 18-23%. Seven hundred ml of 98.2% pure "Medical" helium is added from a graduated cylinder with a leveling bottle to 4,800 ml of air in the system. When this is mixed by the blower, the concentration is 12.5%. The meter reading is compared with the calibration curve to assure proper function of the indicating unit. Two hundred ml of oxygen are then introduced to approximate the concentration of air.

The subject, who has rested for one half hour in the position in which the determination is to be carried out, is now connected without regard to the phase of respiration he is in. At the same time, oxygen is allowed to enter the apparatus at a rate equal to the oxygen consumption of the subject. A respiratory tracing is taken during the seven-minute rebreathing period. At the end of this period the subject is disconnected, and the oxygen flow turned off. The circulation of gases in the closed system is continued until carbon dioxide absorption is complete. The location of the respiratory tracing with reference to the spirometer scale, the meter reading, and the temperature are all recorded.

Gain or loss of oxygen will be evident from the tracing. The meter reading is corrected for this change in oxygen concentration

¹ Van Slyke, D. D., and Binger, C. A. L., *J. Exp. Med.*, 1923, **37**.

² McMichael, J., *Clinical Science*, 1939, **4**, 167.

³ Christie, R. V., *J. Clin. Invest.*, 1932, **11**, 1099.

* Cambridge Instrument Company.

and the final percentage of helium read from the calibration curve. The calculation follows that for McMichael's hydrogen method: If $X + B$ is a volume given by the equation:

$$X + B = \frac{\text{Initial quantity of helium}}{\text{Final concentration of helium}}$$

and if B is the volume of the spirometer system at the end of a normal expiration, then X is the volume of the lung at mid-capacity.

Several corrections must be applied to X . About 10 ml of helium is taken up by the body in the 7-minute period, equivalent to an apparent increase of 110 ml in a pulmonary capacity of 2 liters. The dead space of the mouthpiece and valve was 38 ml. It may be calculated that there is an apparent increase of 30 ml in the capacity because of the difference in inert gas concentration between the lung and spirometer, if one assumes average values of 0.8 for the R.Q. and 2 liters for the mid-capacity. Very little error is introduced by subtracting the rounded total of these 3 figures from X found as above. The correction for temperature and vapor pressure differences is made in the usual way.

The volume at mid-capacity of the lungs of 5 normal subjects was determined by the Christie method,³ by Lassen, Cournand, and Richards' modification of the Christie method,⁴ and by the helium method with the same spirometer system and in duplicate. The error for the 3 methods was calculated and corrected for errors of distribution due to the small size of the sample at a level of significance of $P = 0.05$.^{5, 6} It was found to be ± 0.18 liters, ± 0.22 liters, and ± 0.14 liters respectively. The helium figure averaged 292 ml less than the Christie method, and 202 ml lower than the modified Christie method. This represented a mean decrease of 14.1% and 10.2% respectively, with a probability between 0.98 and 0.99 that these means were different from zero.⁶

The great advantage of a procedure employing a gas, for which analysis by the thermal conductivity method is accurate, lies in the ease and rapidity with which analyses can be made. The advantage of using helium instead of hydrogen is the elimination of the dangers of explosion and arsenic poisoning. Because of the accurate location of the breathing level, the closed method is preferred to

⁴ Lassen, H. C. A., Cournand, A., and Richards, D. W., Jr., *J. Clin. Invest.*, 1937, **16**, 1.

⁵ Mainland, D., *The Treatment of Clinical and Laboratory Data*, Oliver and Boyd, London, 1938, p. 324.

⁶ Fisher, R. A., *Statistical Methods for Research Workers*, Oliver and Boyd, London, 1938, 7th Ed., Chap. V.

open methods. The high diffusibility of helium should result in greater accuracy in determining mid-capacity in subjects with pulmonary emphysema or obstruction, since it is more likely to be uniformly distributed in the lung. There should be less disturbance of normal pulmonary function with this method than with others, because the oxygen concentration of the respiratory mixture is atmospheric.

The method should not be regarded as fully developed. Further consideration must be given to the quantity of helium taken up during the rebreathing, to the correction for oxygen concentration, and to the true relation of spirometer helium concentration to alveolar helium concentration.

11962 P

Effect of Glutathione on Tyrosinase and the Significance of the Dopa Reaction.*

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Bloch,¹ as a result of his discovery of the "dopa reaction" and because it had been impossible to demonstrate tyrosinase in the skin of any of the slightly pigmented vertebrates, postulated the existence of an enzyme that accelerated the oxidation of dihydroxyphenylalanine. He named it "dopa oxidase", and claimed that this enzyme was responsible for melanin formation in human skin. Such an enzyme has not been isolated and its existence has been questioned many times. The controversy in regard to this has been discussed in comprehensive reviews.^{2, 3}

Recently it has been shown⁴ that the activity of the enzyme tyrosinase is regulated by the oxidation-reduction potential of substances

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† Rockefeller Foundation Fellow, 1940-1941.

¹ Bloch, Br., *Z. f. physiol. Chem.*, 1916, **98**, 226.

² Franke, W., in von Euler's *Chemie der Enzyme*, 1934, part 2, p. 38, J. F. Bergmann, München.

³ Oppenheimer, *Die Fermente und ihre Wirkung*, 5th ed., 1926, vol. 2 and supplement, 1939, George Thieme, Leipzig.

⁴ Figge, F. H., *J. Cell. and Comp. Physiol.*, 1940, **15**, 233.