of gestation. No consistent changes were observed in the fatty acids of the neutral lipids between mature and immature fetuses. The stability index also increased toward term. These qualitative and quantitative changes observed during gestation in fetal lung extracts suggest the differentiation of the lung, which is possibly due to the maturity of alveolar cells.

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1. Pattle, R. E., Physiol. Rev., 1965, v45, 48.
2. Klaus, M. H., Clements, J. A., Havel, R. J., Proc. Nat. Acad. Sci., 1961, v47, 1858.
3. Clements, J. A., Physiologist, 1962, v5, 11.
4. Pattle, R. E., Nature, 1961, v189, 844.
5. ———, J. Path. & Bact., 1961, v82, 333.
6. Buckingham, S., Avery, M. E., Nature, 1962, v193, 688.
7. Adams, F. H., Fujiwara, T., J. Pediat., 1963, v63, 337.
8. Orzalesi, M. M., Motoyama, E. K., Jacobson, H. N., Kikkawa, Y., Reynolds, E. O. R., Cook, C. D., Pediat., 1965, v35, 373.
9. Avery, M. E., Mead, J., AMA J. Dis. Child., 1959, v97, 517.
10. Adams, F. H., Fujiwara, T., Emmanouilides, G., Scudder, A., J. Pediat., 1965, v66, 357.
11. Adams, F. H., Moss, A. J., Fagan, L., Biologia Neonat., 1963, v5, 151.
12. Rouser, G., O'Brient, J. S., Heller, D., J. Am. Oil Chemists' Soc., 1961, v38, 14.
13. King, E. J., Biochem. J., 1932, v26, 292.
14. Metcalfe, L. D., Schmitz, A. A., Anal. Chem., 1961, v33, 363.
15. Morrison, W. R., Smith, L. M., J. Lipid Res., 1964, v5, 600.
16. Schaefer, K. E., Avery, M. E., Bensch, K., J. Clin. Invest., 1964, v43, 2080.
17. Clements, J. A., Hustead, R. F., Johnson, R. P., Gribetz, I., J. Appl. Physiol., 1961, v16, 444.
18. Brown, E. S., Fed. Proc., 1962, v21, 438.
19. ———, Am. J. Physiol., 1964, v207, 402.
20. Finley, R. N., Morgan, T. E., Fialkow, H. C., Huber, G. L., Fed. Proc., 1964, v23, 156.
21. Kuenzig, M. C., Hamilton, R. W., Jr., Peltier, L. F., J. Appl. Physiol., 1965, v20, 779.
22. Campiche, M. A., Gautier, A., Hernandez, E. L., Reymond, A., Pediatrics, 1963, v32, 976.
23. Shah, D. O., Schulman, J. H., J. Lipid Res., 1965, v6, 341.
24. Scarpelli, E. M., Gabbay, K. H., Kochen, J. A., Science, 1965, v148, 1607.

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Micromethod for Determination of Specific Gravity of Tissues. (31050)

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During a study of fatty changes taking place in the liver, kidneys, and heart of a large group of fasted mice, it was noted that the specific gravity of these organs was less than those of fed mice. Hence, a rapid method for estimation of the specific gravity of these organs was needed. Since the heart samples available averaged only 25 mg, a micromethod was required. This eliminated the application of Archimedes principle used by Tsai and Lin(1) and by Saathoff(2) by which organs were weighed first in air and and then in saline solution or in water. Attaching a piece of tissue weighing only a few milligrams to a wire and determining the loss of weight in water was not practicable.

Other investigators tried flotation methods using aqueous solutions or hydrocarbon mixtures. Phillips, Van Slyke *et al*(3) adapted the flotation method to blood and plasma and utilized copper sulfate (c.s.) solutions of known specific gravity for suspending the droplets. By using a protein-coagulating medium, dispersion of material from the blood was minimized. Morales, Rathbun, and Smith(4) used this method for determining

the specific gravity of muscle but details were not given.

Initial tests indicated that this was a promising micromethod for determining the specific gravity of tissues. However, little was known of the factors influencing the determinations such as buoyancy effect of air bubbles, size of tissue pieces, drying out of tissues after dissection, constancy of specific gravity of replicate samples of a given organ, stability of the standard solutions, and finally, temperature changes. This study attempted to answer these questions.

Methods. Thirty-six stock solutions of c.s., covering the range from 1.030 to 1.100 at nominal intervals of 0.002, were prepared by dilution of a c.s. solution having a specific gravity of 1.100 at the existing room temperature (72°F)(3). These stock solutions were stored in closed polyethylene bottles and were checked with precision hydrometers which had been calibrated against solutions whose specific gravities were determined with a pyknometer. The agreement between nominal and actual values was generally within 0.001.

Working solutions for use with tissues were kept in 150 mm × 25 mm screw-capped test tubes and were used within ± 5°F of the temperature at which they were prepared (72°F). Each tube was filled about three-quarters full (cir. 35 ml) and care was taken to hold the tube by the neck to avoid temperature changes. A label was attached to each tube for recording each addition of tissue deposited on the bottom. Tissue which floated on the top was always removed but was not recorded.

In use, a piece of tissue weighing between 2 and 6 mg was grasped with previously-dried, stainless steel forceps* and plunged beneath the surface of one of the standard solutions, shaking it quite vigorously several times before releasing it. If the tissue floated to the surface, it was seized and shaken once more to insure freedom from attached air bubbles. If the tissue sank slowly another piece was tested in a solution 0.004 unit heavier, or, if it rose slowly, in a solution 0.004 unit lighter. The tissue was assigned a specific gravity corresponding to that of the solution in which the sample showed neutral buoyancy, that is, when the sample neither rose, nor sank in the liquid. With only 3 or 4 bits of tissue available,† it was generally possible to estimate the specific gravity within 0.001 unit in 3 or 4 trials. It was necessary to judge the buoyancy effect within 15-30 seconds since the tissues became heavier on prolonged immersion.

The effects of the previously-mentioned factors on the determinations of specific gravity were investigated using livers and kidneys of mice. In addition, a comparison was made of values obtained by the c.s. method with those obtained by the classical Archimedes method(1,2).

Results. Using the c.s. method, 5 determinations made on samples of liver taken from different parts of the organ gave a mean value of 1.0942 ± 0.0004 (standard deviation), the range being only 0.001. In another series, made on 7 samples from the same liver, but using samples weighing between 0.5 mg and 6 mg, the mean specific gravity was 1.0941 ± 0.0004, the range again being 0.001. The effect of air bubbles was seen in a series of 5 determinations on the same liver. In these, in which the tissue was not shaken with the forceps after immersion in the c.s. solution, a mean value of 1.0904 ± 0.0021 was found, the range being 0.005. With samples of kidney tissue weighing about 2 mg and exposed to the air, the specific gravity was found to increase from 1.074 at zero time to 1.082 after 20 minutes.

The specific gravity of 35-80 mg portions of liver used in the above experiments was determined by attaching the tissue to a wire 0.005 inch in diameter, suspended from a balance, and weighing both wire and tissue in air and water (Archimedes method). The mean of 5 determinations was found to be 1.0908 ± 0.0076. The range of 0.016 was much greater than that found with the c.s. method. This was due in part to the effect of surface tension on the wire. The change in weight of the tissue during weighing it in air

* Unplated iron or steel forceps are attacked by copper sulfate solutions and should be avoided.

† The tissue should be kept in a moist chamber to avoid loss of moisture.

and in water, due to dehydration and hydration, respectively, added further to the uncertainty of this method.

On increasing the temperature of c.s. solutions 5°F above that at which they were standardized, the specific gravity values were found to remain constant within 0.001. Even when the tissues had been stored in the ice box, equilibrium was attained so quickly when 2 mg samples were tested that no appreciable change in specific gravity could be detected.

The specific gravity of c.s. solutions used for 25-50 tests was found to remain constant within 0.001 for a week. However, the specific gravity of a solution increased after prolonged contact with the tissue and the color of the solution changed from blue to green. A periodic check of the specific gravity of the working solutions should be made with a hydrometer. The standard solutions, stored in stoppered bottles, should maintain their values indefinitely.

Conclusions. The c.s. method for determination of specific gravity of fresh tissue requires only small samples, and is precise, quick and simple to use. With proper precautions against air bubbles and loss of moisture from tissue by exposure to air, this method avoids most of the difficulties inherent with Archimedes method and is more precise. It should find application not only for determining the specific gravity of organs of experimental animals but also for rapid detection of fatty livers by means of biopsy samples(5).

Summary. The copper sulfate method of determining the specific gravity of blood has been applied to fresh tissues. A number of factors influencing the determinations have been studied. Comparison of this method with that based on Archimedes principle have shown the copper sulfate method to be decidedly superior.

1. Tsai, C., Lin, C., Chinese J. Physiol., 1939, v14, 39.

2. Saathoff, J., Monat. f. Veterinärmedizin, 1947, v2, 191.

3. Phillips, R. A., Van Slyke, D. D., Dole, V. P., Emerson, K., Hamilton, P. B., Archibald, R. M., Bumed News Letter, U. S. Navy, 1943, v1, 1.

4. Morales, M. F., Rathbun, E. N., Smith, R. E., Pace, N., J. Biol. Chem., 1945, v158, 677.

5. Billing, B. H., Conlon, H. J., Hein, D. E., J. Clin. Invest., 1953, v32, 214.

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Edema of the Skin and Menstruation in Monkeys (*Macaca mulatta*) On Repeated Estrogen Treatments.* (31051)

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The development of a sexual skin in adolescent female monkeys *(Macaca mulatta)* and its subsequent maturation into a condition seen in the adult has been described(1). The maturation process involves the loss of an edematous condition responsive to estrogen to one in which the sexual skin is comparatively thin and has a reddish color which may not only include the perineum and buttocks but also extend for various distances down the legs, upon the flanks, and over the symphysis pubis. The pale sexual skin of a castrated juvenile animal becomes greatly swollen when estrogen is given, whereas that of a castrated adult develops a red color and shows little or no edema.

When castrated adult monkeys are given large doses of estrogen a generalized edema of the skin results which may extend over most of the body with the exception of the matured sexual skin of the buttocks and perineum. The skin of the back and sides develops prominent pachydermatous folds (Fig. 1). The face also is usually affected, particularly the supraorbital ridge, and swelling about the eyes may be so great as to partly obstruct vision. Such a condition can be de-

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