amino acids. Under the experimental conditions used in this work, the intracellular concentration of AIB approaches equilibrium exponentially in both types of tissue. Swelling markedly enhances the rate of AIB transfer across the cell membrane and doubles the value of its final distribution in the intracellular water. This increase is still higher if expressed in terms of individual cells. The significance of these observations with respect to the increased incorporation rate of labelled amino acids into protein found in livers with cloudy swelling is discussed.

1. Fonnesu, A., Severi, C., *Science,* 1956, v123, 324.
2. Popjak, G., *J. Path. Bacteriol.,* 1948, v60, 75.
3. Ciaranfi, E., Fonnesu, A., Guidotti, G., *Atti Soc. It. Patol.,* 1957, v5, 521.
4. Riggs, T. R., Walker, L. M., Christensen, H. N., *J. Biol. Chem.,* 1958, v233, 1479.
5. Noall, M. W., Riggs, T. R., Walker, L. M., Christensen, H. N., *Science,* 1957, v126, 1002.
6. Manchester, K. L., Young, F. G., *Biochem. J.,* 1960, v75, 487.
7. Eichhorn, J., Scully, E., Halkerston, I. D. K., Hechter, O., PROC. SOC. EXP. BIOL. & MED., 1961, v106, 153.
8. Kipnis, D. M., Noall, M. W., *Biochim. Biophys. Acta,* 1958, v28, 226.
9. Wool, I. G., *Am. J. Physiol.,* 1960, v199, 715.
10. Christensen, H. N., Parker, H. M., Riggs, T. R., *J. Biol. Chem.,* 1958, v233, 1485.
11. Roe, J. H., Epstein, J. H., Goldstein, N. P., *ibid.,* 1949, v178, 839.
12. Del Monte, U., *Biochim. Biophys. Acta,* 1961, v49, 431.
13. Swan, A. G., Ellington, E. E., Miller, A. T., Jr., *Am. J. Physiol.,* 1960, v199, 1223.
14. Price, J. M., Laird, A. K., *Cancer Research,* 1950, v10, 650.
15. Abercrombie, M., Harkness, R. D., *Proc. Roy. Soc. B.,* 1951, v138, 544.
16. Anderson, N. G., *Science,* 1953, v117, 627.
17. Cramer, H., *Mathematical Methods of Statistics,* Princeton Univ. Press, 1958.
18. Rosenberg, L. E., Blair, A., Segal, S., *Biochim. Biophys. Acta,* 1961, v54, 479.
19. Harkness, R. D., *J. Physiol.,* 1952, v117, 267.
20. Christensen, H. N., in *Membrane Transport and Metabolism,* Kleinzeller and Kotyk, Editors, Academic Press Publ., 1961, 470.
21. Corvie, D. B., McClure, F. T., *Biochim. Biophys. Acta,* 1959, v31, 236.
22. Halvorson, H. O., Cohen, G. N., *Ann. Inst. Pasteur,* 1958, v95, 73.
23. Wright, B. E., Anderson, M. L., *Biochim. Biophys. Acta,* 1960, v43, 67.
24. Kipnis, D. M., Reiss, E., Helmreich, E., *ibid.,* 1961, v51, 519.

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Distribution of Chromatin Bodies in an XX/XY True Hermaphrodite.* (27630)

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The discovery by Barr and Bertram(1) of the chromatin body in the nerve cells of female cats set the stage for the development of nuclear sexing as an important clinical tool. However, the origin of the chromatin body is still not clear although a number of theories have been proposed to explain this feulgen-positive structure found in intermitotic nuclei of most female cells. Barr originally suggested that the chromatin body was due to somatic pairing of the heterochromatic parts of 2 X chromosomes. The observation in domestic chickens(2), in which the female is the heterogametic sex, that the female is chromatin positive and the male chromatin negative was contradictory to Barr's hypothesis, and also raised the possibility that the chromatin body might be a sex influenced character subject to extracelluar factors. Several observations on different intersexual conditions, however, indicate that the chromatin body is more likely cell autonomous. For example, in the testicular feminizing syndrome, where XY cells have been subjected to a female hormonal environment, the cells are chromatin

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negative. In the bovine free-martin a normal frequency of chromatin positive cells(3) has been demonstrated in neuronal tissue even though the animal had been extensively masculinized by its male cotwin. We have recently studied an hermaphrodite who is an XX/XY mosaic in a variety of tissues, and consequently offers a unique opportunity to test further the independence of the chromatin body formation. The chromatin body distribution in various tissues of this hermaphrodite is reported here.

Biopsies of various tissues of this true hermaphrodite(4) who was found to be an XX/XY mosaic resulting from a dual fertilization(5) were established in cultures by growing the cells in a mixture of medium 199 and 20% fetal calf serum. Chromosome studies were done by the method of Ruddle *et al.*(6) and karyotypes prepared. Transfers of the original cultures were grown on glass slides and stained with cresyl violet. The slides were examined for chromatin bodies and percentage of positive chromatin bodies compared to percentage of XX karyotypes found in that tissue.

Table I shows the number of XX and XY chromosome complements found in the cell cultures of the different biopsy specimens. This patient exhibited marked topographical segregation of the 2 cell types. The results of chromatin body determinations of the same cell cultures are also shown in Table I. The control values compare favorably with those reported by Burlington(7), Myers(8), Fraccaro and Lindsten(9) on somatic tissue culture cells.

TABLE I. Results of Chromosome and Chromatin Body Studies in Various Tissues.

Tissue	XX cells	XY cells	% chromatin bodies
Female control		0	43
Male "	0		4
Skin (right abdomen)	3	10	13
Skin (left abdomen)	8	1	36
Ovary	10	0	50
Ovotestis (ovarian part)	9	2	35
Ovotestis (testicular part)	2	12	15
Clitoris	4	7	29

In Fig. 1 the percentage of chromatin bodies to percentage of XX cells found in the various tissues is compared. The high correlation between these two characteristics is evident. There is a direct relationship of percentage of chromatin body cells found to percentage of XX cells found in all tissues except the testicular part of the ovotestis and the clitoris. The lack of correlation of these lat-

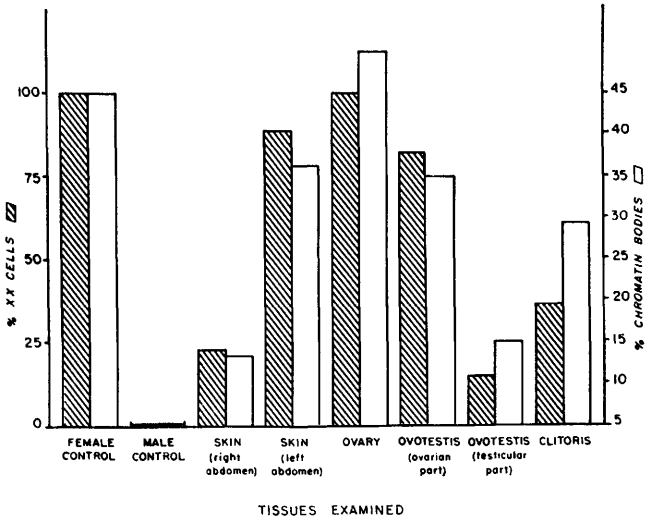

FIG. 1. Relationship of percentage of XX cells to percentage of chromatin bodies found in various tissues.

ter 2 tissues might be due to the small number of cells sampled for karyotyping.

These results indicate that the close proximity of XY cells has no effect on the ability of XX cells to form chromatin bodies. Similarly the close proximity of XX cells does not appear to cause XY cells to form chromatin bodies. This is evident in the 3 tissues containing an excess of XX cells and in one of the tissues containing an excess of XY cells. In the other 2 tissues containing an excess of XY cells, perecentage of chromatin bodies is higher than percentage of XX cells. If this discrepancy were not due to methodological errors, one might think that the presence of XX cells in these tissues stimulated XY cells to form chromatin bodies. The results found in the other 4 tissues make this seem unlikely. Thus further evidence for the cell autonomy of the formation of chromatin bodies is presented.

1. Barr, M. L., Bertram, L. F., *Nature,* 1949, v163, 677.

2. Kosin, I. L., Ishizaki, H., *Science,* 1959, v130, 43.

3. Moore, K. L., Graham, M. A., Barr, M. L., *J. Exp. Zool.,* 1957, v135, 101.

4. Waxman, S. H., Gartler, S. M., Kelley, V. C., *J. Pediat.,* 1962, v60, 540.

5. Gartler, S. M., Waxman, S. H., Giblett, E., *Proc. U.S. Nat. Acad. Sci.,* 1962, v48, 332.

6. Ruddle, F. H., Berman, L., Stulberg, C. S., *Cancer Res.,* 1958, v18, 1048.

7. Burlington, H., *Exp. Cell Res.,* 1957, v16, 218.

8. Myers, L., *J. Path. Bact.,* 1959, v78, 29.

9. Fraccaro, M., Lindsten, J., *Exp. Cell Res.,* 1959, v17, 536.

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Evaluation of the Epinephrine Skin Test as a Biological Assay for Endotoxin.* (27631)

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Biological tests for identification and assay of bacterial endotoxins are few. For various reasons any given test may not be suited to the experimental objective. Among those currently in use is the epinephrine skin test introduced by Thomas, who showed that rabbits given an intravenous injection of 1 to 50 μg of endotoxin develop extensive hemorrhagic necrosis in skin sites injected with 100 μg epinephrine at the same time or up to four hours afterward(1). The test is said to be specific for endotoxin, but Nagler and Zweifach have reported that serotonin, given intravenously, produces the same effect(2). If substantiated, this finding would invalidate the test.

The present study was undertaken to determine: a, the sensitivity and reproducibility of the epinephrine skin test (EST); b, the comparative sensitivity of this test and that of the 10-day-old chick embryo for endotoxin; c, the length of time circulating endotoxin can be detected by each test following a lethal dose of endotoxin intravenously; and d, the specificity of the EST for endotoxin, with special reference to the reportedly similar response to serotonin.

Materials and methods. Animals. Adult albino rabbits weighing 1.5 to 2.5 kg were used.

Endotoxin and serotonin assays by the EST. Endotoxin** or serotonin† was injected into the ear vein of rabbits, followed one hour later by separate injections of 50, 100 and 200 μg of epinephrine‡ into the skin of the abdomen, previously shaved and cleansed with 70% isopropyl alcohol. The skin reac-

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** Endotoxin: Difco #8, *E. coli,* 1 mg/ml, in sterile saline dilutions of 1, 10, and 100 μg/ml.

† Serotonin: Serotonin creatinine sulfate, Nutritional Biochemicals Corp., in sterile saline dilutions of 1, 10, and 100 μg/ml.

‡ Epinephrine: Epinephrine HCl, 1:1000, Lederle.