G. C. de Hevesy, A. G. Forssberg, J. D. Abbatt, Oliver and Boyd, Ltd., Edinburgh, 1957, 197-203.

8. Nowell, P. C., Cole, L. J., Habermeyer, J. G., Roan, P. L., *Cancer Research,* 1956, v16, 258.

9. Amos, D. B., Gorer, P. A., Mikulska, Z. B., *Proc. Roy. Soc., London, s. B.,* 1955, v144, 369.

10. Gorer, P. A., Lyman, S., Snell, G. D., *ibid.,* v135, 499.

11. Gluecksohn-Waelsch, S., Ranney, H. M., Sisken, B. F., *J. Clin. Invest.,* 1957, v36, 753.

12. Smithies, O., *Biochem. J.,* 1955, v61, 629.

13. Makinodan, T., Anderson, N. G., *Blood,* 1957, v12, 984.

14. Snell, G. D., Russell, E., Fekete, E., Smith, P., *J. Nat. Cancer Inst.,* 1953, v14, 485.

15. Counce, S., Smith, P., Barth, R., Snell, G. D., *Ann. Surg.,* 1956, v144, 198.

16. Trentin, J. J., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 688.

17. Uphoff, D. E., *J. Nat. Cancer Inst.,* 1957, v19, 123.

18. Uphoff, D. E., Law, L. W., *ibid.,* 1958, v20, 617.

Received October 1, 1958. P.S.E.B.M., 1958, v99.

Incorporation of Cystine and Lysine by Normal and "Cystinuric" Leukocytes. (24466)

F. F. BECKER AND H. GREEN (Introduced by B. W. Zweifach)
Dept. of Pathology, N. Y. University Bellevue College of Medicine

Cystinuria has been shown by Dent(1,2) to be a disease of renal tubules in which amino acids cystine, lysine, arginine and ornithine fail to be absorbed from the glomerular filtrate. Since these substances can be considered as structurally related di-amino acids(3) and their absorption from the glomerular filtrate is largely controlled by a single gene(4), it has been suggested that a single mechanism for absorption of these amino acids exists in the renal tubules. However, other kinds of body cells are capable of concentrating amino acids, such as muscle(5), white blood cells (6), tumor cells(7), and established cell lines grown *in vitro*(8). If the concentration mechanism of amino acids by such cells were related to that of the kidney tubules, these cells might also be affected in the cystinuric individual, for biochemical defects in genetic diseases are frequently widespread. The fact that such a general metabolic abnormality has not been found in cystinuric subjects does not argue against this possibility, for whereas the defect in kidney tubules results in obvious failure to transport amino acids against a concentraton gradient into the blood, the other body cells might continue to obtain sufficient amino acids from blood by diffusion alone, the blood level of these amino acids in cystinurics remaining normal or only slightly reduced(2,9). If however, such cells were placed in a medium containing a considerably lower amino acid concentration than normal blood, any latent defect of this kind in the cystinuric cells might become evident. In the present experiment, therefore, white blood cells of normal and cystinuric subjects were compared in their abilities to incorporate lysine and cystine from the medium, at concentrations below those existing normally in human blood, and at which rate of uptake depends upon concentration in the medium. At the lowest concentration tested, uptake of amino acids by normal and "cystinuric" leukocytes was the same.

Methods. The cystinuric subjects were a brother and sister in their forties who had an extensive family history of the disease; each had been operated on more than once for renal stones, but otherwise was in good health. Their urine gave a strong cyanide-nitroprusside test for cystine. White blood cells were separated from freshly drawn human blood by fibrinogen sedimentation as described by Skoog and Beck(10). They were resuspended in isotonic sodium chloride solution and washed twice. The final suspension was made in the balanced salt solution of Eagle, BSS,(11) and the cells incubated with gentle shaking in water bath at 37°C, under atmosphere of 5% CO_2, 95% O_2. At time zero, labeled amino

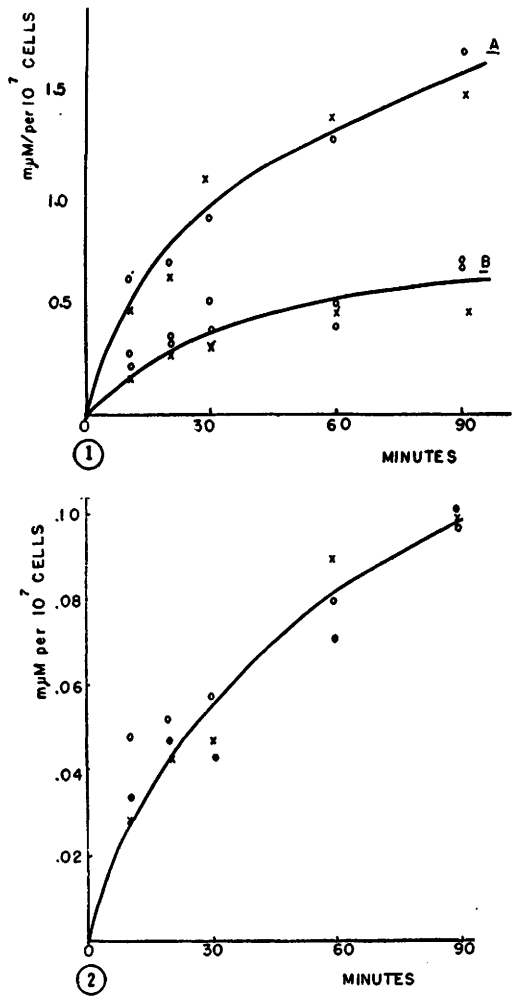

FIG. 1. Lysine incorporation into normal and cystinuric leukocytes. Medium concentration of 2-C^{14} DL-lysine (0.6 mC/mM). Curve A—2.2 × 10^{-4} molar. Curve B—0.43 × 10^{-4} molar. ○—normal leukocytes. ×—cystinuric leukocytes. Ordinate represents mμmoles of amino acid taken up by the cells.

FIG. 2. Cystine incorporation into normal and cystinuric leukocytes. Medium concentration of S^{35} L-cystine (3.84 mC/mM) 7.1 × 10^{-6} molar. ●—normal leukocytes in absence of lysine and arginine; ○—normal leukocytes and ×—cystinuric leukocytes, each in presence of 2.5 × 10^{-3} molar L-lysine and L-arginine.

acid was added, and at intervals aliquots were diluted into 20 ml cold BSS and plated and washed on a membrane filter (millipore). The infinitely thin samples were air dried, mounted on planchets, heated in forced draft oven at 70°C, one hour, and then counted. (Previous experiments had shown that substantially all the counts at these concentrations were insoluble in hot 5% trichloroacetic acid, and therefore had become incorporated into cell protein.) From counts/minute in the sample, and the specific activity of amino acid added, the molar quantities taken up were calculated.

Results. Fig. 1 shows uptake of lysine at 2 different concentrations in the medium.

Incorporation began immediately at maximum rate but slowed considerably by 90 minutes. At these low concentrations of lysine, rate of uptake depended on concentration, as previously shown for leucine uptake by white blood cells(12). Rate of uptake at a DL-lysine concentration of 0.43 x 10^{-4}M was only about half that at 2.2 x 10^{-4}M, but at both concentrations the cystinuric white blood cells took up lysine as well as normal ones.

Uptake of cystine was followed at concentrations as low as 7.1 x 10^{-6}M and the white blood cells of cystinuric subjects took up cystine at a normal rate. Lysine and arginine added to the medium in high concentrations (2.5 x 10^{-3}M) did not affect rate of uptake of cystine by either normal or cystinuric white cells (Fig. 2).

It appeared, therefore, that no defect could be demonstrated in uptake of lysine and cystine by cystinuric white blood cells, at concentrations considerably below normal blood concentration(9). In addition, there was no demonstrable effect of arginine or lysine on cystine uptake by either cystinuric or normal white blood cells, although such competition occurs in the renal tubules(13). If, as seems likely, cellular concentrating mechanisms for amino acids play a role in determining rate of entry of amino acids into cells(14) then these mechanisms may be basically different from those involved in tubular absorption of amino acids from the glomerular filtrate, or, if they are similar, a defect in such a mechanism might exist in tubular cells alone.*

Summary. Leukocytes of cystinuric patients incorporate labelled cystine and lysine from the medium at a normal rate, even when

* It is interesting to note that in a recent study of the concentrating power of human cells cultivated *in vitro* (HeLa) the amino acids involved in cystinuria formed a distinct group as the most poorly concentrated of all the amino acids(8).

these amino acids are added in very low concentration. Rate of uptake of cystine by either normal or "cystinuric" leukocytes is not affected by presence of high concentrations of arginine and lysine. The mechanism of renal tubular concentration may therefore be different in kind from that of leukocytes.

1. Dent, C. E., Heathcote, J. G., Joron, G. E., *J. Clin. Invest.*, 1954, v33, 1210.
2. Dent, C. E., Senior, B., Walshe, J. M., *ibid.*, 1954, v33, 1216.
3. Dent, C. E., Rose, G. A., *Quart. J. Med.*, 1951, v20, 205.
4. Harris, H., Robson, E. B., *Am. J. Med.*, 1957, v22, 774.
5. Christensen, H. N., Streicher, J. A., *Arch. Biochem.*, 1949, v23, 96.
6. Borsook, H., Deasy, C. L., Haagen-Smit, A. J., Keighley, G., Lowy, P. H., *J. Biol. Chem.*, 1950, v186, 297.
7. Christensen, H. N., In *Amino Acid Metabolism*, 1955, p63, W. D. McElroy and B. Glass, Eds., Johns Hopkins Press.
8. Piez, K. A., Eagle, H., *J. Biol. Chem.*, 1958, v231, 533.
9. Stein, W. H., Moore, S., *ibid.*, 1954, v211, 915.
10. Skoog, W. A., Beck, W. S., *Blood*, 1956, v11, 436.
11. Eagle, H., *Science*, 1955, v122, 501.
12. Baker, W. H., Zamecnik, P. C., Stephenson, M. L., *Blood*, 1957, v12, 822.
13. Doolan, P. D., Harper, H. A., Hutchin, M. E., Alpen, E. L., *Am. J. Med.*, 1957, v23, 416.
14. Heinz, E., *J. Biol. Chem.*, 1957, v225, 307.

Received October 1, 1958. P.S.E.B.M., 1958, v99.

Effects of Handling on the Adrenalectomized Rat.* (24467)

H. ELRICK, L. BERNSTEIN, E. DIAMANT, J. M. WHITEHOUSE AND D. BORDA-BOSSANA
(Introduced by Gordon Meiklejohn)
Medical Research Labs. and Depts. of Psychology, V. A. Hospital, Medicine and Psychology, University of Colorado School of Medicine, Denver.

Previous studies using normal young albino rats have shown that handling or gentling results in significant increases in weight gain and skeletal length(1,2), superior learning ability(3), and increased resistance to certain forms of stress(4,5). It has recently been shown that hypophysectomy alters some of the response to handling(6). The purpose of this paper is to report the influence of adrenalectomy on certain effects induced by handling.

Methods. Three consecutive experiments were done using young male rats of Sprague-Dawley strain.† Bilateral adrenalectomy was performed in a single operation on 23rd day of life in Exp. I and on 21st day in Exp. II and III. The animals were weighed and then divided into 2 equal groups: 1) Beginning on day following adrenalectomy each animal of experimental group was handled individually for 10 minutes every day at same time of day for 2 months. In each experiment a different investigator handled the animals. Handling consisted of holding the animal in one hand and stroking it repeatedly with the other hand from head to tail with animal facing the handler. 2) Control animals were never touched after their original placement in cages following adrenalectomy. A weighed amount of food in dish (Exp. I and II) or special feeding cup‡ to minimize spillage (Exp. III) was placed in each cage and leftover food weighed to determine individual daily food consumption. Food used in Exp. No. I and II was as follows: 480 g canned dog food (Pard®), 2 slices of crumbled whole wheat bread, and 2.5 ml Vi-Daylin® (liquid multivitamin preparation marketed by Abbott Labs.). These ingredients were thoroughly mixed and formed a soft, adherent mass such that food loss from spillage was minimal. In Exp. III powdered Purina Rat Chow was used. In all experiments 5% glucose in normal saline was supplied as drinking water. Animals were housed

* Supported by grant from U.S.P.H.S.
† Obtained from Hormone Assay Labs., Chicago.
‡ Obtained from A. S. Aloe and Co.