perature. 200-gm. female rats were taken from an atmosphere varying from 75° to 80° F. and placed on a metal surface in a cold room maintained at 36–38° F. Animals of exps. 1–3 were injected with 50 mgm./kgm. sodium heparin intraperitoneally to facilitate blood collection, 30–40 min. before the beginning of exposure to cold. At specified intervals, of 15 sec.–20 min. duration, they were decapitated, and bled into cold siliconized test-tubes containing 1 drop of heparin. Adapting a method of Weissbach[6], 0·5 c.c. rat blood was pipetted into 7 c.c. of cold distilled water and lysed, and 2·5 c.c. of a 5 per cent zinc sulphate solution was added with shaking. The tubes were then neutralized with the appropriate quantity of sodium hydroxide, shaken again and centrifuged. The supernatants were treated in one of three ways: they were analysed for 5-hydroxyindole content by direct fluorimetry[6], or extracted and analysed specifically for 5-hydroxytryptamine (serotonin) by combination of the zinc sulphate protein precipitation[6] with the Bogdanski extraction procedure[7], one very close to Weissbach's extraction method, or, they were split, and both direct and extraction analyses carried out on the same sample. The fluorimetric readings of samples and recoveries were performed on a Farrand spectrophotofluorimeter. Samples were found to have emission and absorption spectra identical with processed serotonin.

Table 1. Effect of Cold Exposure on Whole Blood Serotonin

Exp.	No. of samples	Duration cold exposure	Whole-blood γ/c.c. 5-hydroxyindole		P
			Direct method	Extraction method (serotonin)	
1 Rat (heparin)	5	0 min.	0·94 ± 0·24	0·43 ± 0·09	< 0·01
	4	5–7 ,,	1·77 ± 0·20	1·08 ± 0·22	
	4	9–11 ,,	1·63 ± 0·33	1·08 ± 0·17	
	4	20 ,,	1·44 ± 0·50	0·72 ± 0·23	
2 Rat (heparin)	16	0 min.	0·95 ± 0·21	—	< 0·001
	18	15–75 sec.	1·39 ± 0·24	—	
	8	2 min.	1·40 ± 0·34	—	
	8	4 ,,	1·09 ± 0·26	—	
	5	6 ,,	1·01 ± 0·12	—	
	5	11 ,,	0·94 ± 0·28	—	
3 Rat (heparin)	3	0 min.	1·95 ± 0·67	1·77 ± 0·88	—
4 Rat (no heparin)	10	0 min.	—	1·52 ± 0·46	< 0·01
	10	5–9 ,,	—	2·20 ± 0·49	
5 Rabbit	7	0 min.		3·29 ± 0·86	—
Human	7	0 ,,	—	0·12 ± 0·06	
Rat	8	0 ,,	—	1·41 ± 0·29	

The results are described in Table 1. Exp. 1 depicts increases in blood 5-hydroxyindole and serotonin that are approximately equivalent, about 0·7γ serotonin/c.c. whole blood. This elevation begins falling away between the 11th and 20th minute. Exp. 2 examines the same phenomenon by the direct method only. In this experiment the rise and fall of blood 5-hydroxyindole follows a more rapid time course. Exps. 3 and 4, performed a month later, demonstrate an equivalent cold-induced serotonin elevation in animals not injected with heparin. In these experiments, the normal values are seen to be somewhat high in comparison with the values obtained in other experiments for the rat[8,9]. In contrast, as shown in Exp. 5, human and rabbit bloods analysed simultaneously with rat samples gave serotonin titres similar to those obtained by other methods[6,8–11]. No other laboratories report whole-blood analyses for the rat, all blood serotonin values for rats reported to date being derived by calculation from analysis of platelets or platelet-rich plasma[6,8–11]. Thus the somewhat higher normal values we observe remain to be explained: they may reflect a seasonal variation, an inefficiency of methods for gathering platelets in the rat, or, possibly, the existence of a non-platelet source of blood serotonin in this species.

In contrast to Toh's observations[3], it is important to note that the alterations we have observed could not have been provoked by a non-specific lowering of body temperature. Rectal temperatures were not significantly altered in rats exposed to cold for 30 min.

The question of the source of the increase in blood serotonin and the relation of this observation to a local and general increased serotonin metabolism are under investigation at present. We speculate that the perturbations in serotonin concentration observed indicate serotonin participation in cardiovascular and/or metabolic adjustments to cold.

Note added in proof. In recent experiments employing blood drawn by cardiac puncture a cold-induced increase in plasma serotonin was not observed.

This research was supported by U.S. Public Health grant *A*–4064 *Met.*

Paul Gordon*

Department of Pharmacology,
Northwestern University,
Medical School,
Chicago, Illinois.

* Present address: Department of Pharmacology, The Chicago Medical School, Chicago, Illinois.

1 Erspamer, V., *Pharm. Rev.*, **6**, 425 (1954).
2 Towne, J. C., and Sherman, J. O., *Proc. Soc. Exp. Biol. Med.*, **103**, 721 (1960).
3 Toh, C. C., *J. Physiol.*, **151**, 410 (1960).
4 Towne, J. C. (personal communication).
5 Lee, C. Y., *J. Physiol.*, **152**, 405 (1960).
6 Weissbach, H., Waalkes, T. P., and Udenfriend, S., *J. Biol. Chem.*, **230**, 865 (1958).
7 Bogdanski, D. F., Pletscher, A., Brodie, B. B., and Udenfriend, S., *J. Pharm. Exp. Ther.*, **117**, 82 (1956).
8 Erspamer, V., *J. Physiol.*, **133**, 1 (1956).
9 Humphrey, J. H., and Jaques, R., *J. Physiol.*, **124**, 305 (1954).
10 Udenfriend, S., and Weissbach, H., *Fed. Proc.*, **13**, 412 (1954).
11 Erspamer, V., and Faustini, R., *Naturwiss.*, **40**, 317 (1953).

Effect of Sodium Fluoride on Calcium Metabolism of Human Beings

Although bones of human subjects become abnormally dense after several years of industrial exposure to fluorides[1,2], no work on the effect of fluoride on calcium balance of human beings has been carried out. Roholm[1] and others[2] have shown that the severity of skeletal changes is correlated approximately with the extent and duration of exposure to fluoride: moderate exposure resulting in increased density of otherwise grossly normal bone, but prolonged heavy exposure resulting in marked changes, including periosteal bone-formation and calcification of ligaments and tendons. How such complex skeletal effects are brought about is unknown; however, the microscopic appearance of depressed or absent osteoclastic activity in bones of moderately affected human beings[1] suggests that relatively low doses of fluoride may somehow depress resorption of bone.

©1961 Nature Publishing Group

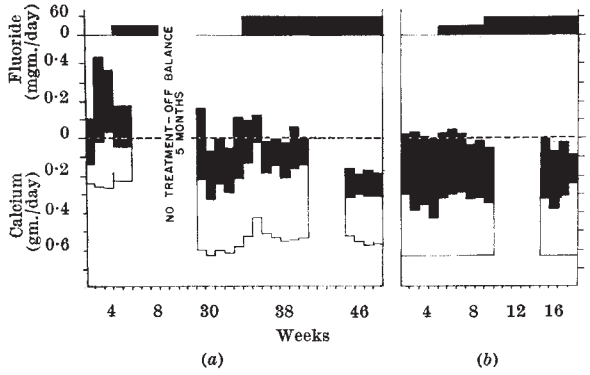

Fig. 1. Calcium balances of a patient with widespread and active Paget's disease (*a*) and of a patient with inactive post-menopausal osteoporosis (*b*). Data are plotted after Albright with intake down and urinary above fæcal calcium

Because of the demonstrated skeletal effect of fluoride in human beings, and because of the relatively benign nature, even of advanced fluorosis[1], it is possible that fluoride could be of use in treatment of diseases where skeletal mass is reduced, the rate of bone resorption accelerated, or both. This work was carried out to determine whether or not metabolic effects, particularly retention of calcium, could be observed during treatment of human beings with sodium fluoride. Six patients with osteoporosis and one with Paget's disease were given carefully measured diets which contained approximately constant amounts of calcium, phosphorus and nitrogen. After a preliminary period of at least one week, stools and urine were collected and balance measurements carried out. Control observations were made, usually for three weeks, after which the patients were given sodium fluoride orally in divided doses (all other treatments remaining unchanged). In early work small doses of fluoride were used and treatment was given for only a few weeks. As it was found that the medication was well tolerated, and that effects observed were seen only after several weeks, the dose was increased to 60 mgm. fluoride a day and duration of treatment extended to 14 or more weeks.

Changes in balance of calcium (the analysis[3] for calcium was unaffected by addition of sodium fluoride) of a subject with widespread and progressive Paget's disease are illustrated by Fig. 1*a*. The decreased rate of excretion of calcium in urine and the calcium retention were most marked late in the investigation, and did not appear to have become maximal during the period of observation. Fig. 1*b* shows similar but less-striking results from study of a post-menopausal patient with minimal X-ray evidence of osteoporosis. Other subjects with osteoporosis also responded by positive change in calcium balance and decreased rate of excretion of calcium in urine. (In one, with osteoporosis secondary to treatment of rheumatoid arthritis with prednisone, urinary calcium excretion fell from 204–240 mgm. per day to 24–68 mgm. per day from the twelfth to the fifteenth week of treatment. This was accompanied by increase of about 80 mgm. a day of fæcal calcium; the intake remaining constant.)

Other significant changes were, in several osteoporotic patients, increased alkaline phosphatase activity in plasma, from normal pre-treatment values to 16–38 King–Armstrong units per 100 ml., decreased rate of excretion of citrate and accelerated excretion of acid mucopolysaccharides in urine. No significant alteration occurred, during treatment of any patient, of concentration in plasma of calcium, phosphorus or citrate. No change in X-ray appearance of the skeleton or abnormality of hæmatological, renal or hepatic function could be measured. The only evidence of possible untoward effect was that, during the period of treatment, two of the seven subjects developed sub-acromial bursitis.

Although these results do not show whether or not fluoride will be useful in treatment of human subjects, they do demonstrate profound effects of fluoride ion on calcium metabolism of patients with several skeletal diseases.

This work was supported in part by a grant (*A*–3347) from the National Institutes of Health, U.S. Public Health Service.

CLAYTON RICH*
JOHN ENSINCK†

The Rockefeller Institute,
New York.

* Present address: Veterans Administration Hospital, Seattle, Washington.

† Present address: University of Washington School of Medicine, Seattle, Washington.

[1] Roholm, K., *Fluorine Intoxication* (H. K. Lewis Co., Ltd., London, 1937).

[2] Greenwood, D. A., *Physiol. Rev.*, **20**, 582 (1940).

[3] Rich, C., *J. Clin. Endocrinol. and Metabol.*, **20**, 147 (1960).

PHARMACOLOGY

Effect of Serotonin on Absorption and Distribution of Calcium-45 in the Rat

CERTAIN pharmacological effects of serotonin might be related to its action on the permeability of cell membranes to various ions. It has been shown that serotonin inhibits the pigment effusion, sodium uptake and potassium efflux from slices of red beetroots *in vitro*[1]. The loss of potassium from erythrocytes in cold storage is reduced by serotonin[2]. An increase of the efflux of sodium and potassium from the isolated skin of a frog was also shown in the presence of serotonin[3]. This latter effect was not considered to be the result of non-specific damage of the cell membranes, because the permeability to Evans blue or sucrose was not affected by serotonin[3]. It is, however, well known that serotonin exerts a marked effect on the capillary permeability in rats[4–6]. Following a different approach, Woolley has proposed that there is an interaction between serotonin and calcium in isolated organs on the basis that serotonin no longer stimulates the uterus in the presence of a calcium-chelating agent[7]. This finding was interpreted as an effect of serotonin in facilitating the penetration of calcium into the cells[8]. On the other hand, calcium seems to be an important factor for serotonin release during the antigen–antibody reaction[9,10].

These results prompted us to investigate a possible role of serotonin in the absorption and distribution of calcium. Experiments have been carried out *in vitro* on the everted intestinal sac of the rat, according to the method of Wilson and Wiseman[11]. Serotonin (5-hydroxytryptamine creatinine sulphate) at a concentration of 20 μgm./ml. increased the absorption of calcium-45 (after 15, 30 or 60 min. of incubation), while acetylcholine (20 μgm./ml.) had no effect. Creatinine sulphate (20 μgm./ml.) was also ineffective. Serotonin retained its effect when the everted intestinal sac was incubated at 5° C. Some of the results are summarized in Table 1.

©1961 Nature Publishing Group