in evoking release. A feed-back effect at pituitary level has been demonstrated by direct micro-injection of thyroxine into the pituitary (13,14). Yamada and Greer(14) and Yamada(15) have, furthermore, demonstrated an inhibitory effect on TSH release by micro-injection of thyroxine into anterior hypothalamus of the rat. Integrity of these hypothalamic areas is, however, not essential for feed-back inhibition since thyroxine is still effective after electrolytic destruction of anterior hypothalamus(16,17). A possible role of other brain areas is suggested by Mess(18-20) who demonstrated that the depressing effect of thyroxine upon thyroid weight and pituitary TSH content was notably modified by lesions of the habenula complex.

Summary. Analysis of time sequence in pituitary and thyroid phenomena involved during acute response of hamsters to cold exposure indicates a rapidly activated neural component. Within 0.5-1 hour after cold, sensory perception of this temperature change has generated sufficient input into hypothalamic effector mechanisms to deplete pituitary TSH by 60%. After a latent period of 1.5 hours, accelerated release begins and results in 25-30% decrease of thyroidal I^{131} during 12 hours of cold. Thyroxine (10 μg) is capable of inhibiting thyroidal I^{131} release when administered as long as 2-3 hours after exposure to cold.

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Cholesterol Concentration in Human Serum and Blood Cells.* (25841)

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The concentration of cholesterol in human erythrocytes has been reported to be much less variable than in plasma and relatively independent of the latter value(1,2). However, the data of Foldes and Murphy(3) from 20 "normals" aged 19 to 35 and 20 surgical patients aged 70 to 90 indicate considerable variation between individuals, the standard deviation of red cell concentration in the normals being 27.6 mg per 100 ml, or 16% of the mean of 173.0. The variability was equally large when the 2 age groups were separately considered. Moreover, when plasma cholesterol changes were induced by treatment of 17 thyroid patients the data reported indicate cell cholesterol concentration changes in the

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same direction as those in the plasma(4). Cell concentration in these studies was calculated from measurements of the hematocrit and concentrations in the whole blood and in the plasma. The present paper reports the findings from both indirect and direct methods of estimating cholesterol in bloods from a sample of men and from men in dietary experiments which induced large changes in the serum cholesterol level.

Methods and procedures. Casual venous blood samples were drawn from 38 men, mostly ostensibly healthy but including some patients with coronary heart disease. Ages ranged from 28 to 65 years, the mean being 57.4 years. Six schizophrenic men, aged 40 to 57 years, long resident at the Hastings State Hospital, were studied in connection with controlled dietary alterations. After the men were stabilized on a diet fixed as to kind and amount of fat, the diet was changed by substituting, isocalorically, carbohydrates and poly-unsaturated fats for most of the saturated fats or vice versa. Periods of 3 weeks on each diet were used and blood samples were drawn on the last 2 days on each diet. Plasma and whole blood cholesterol was estimated in duplicate 0.1 ml samples by the method of Abell, *et al.*(5) as modified by us (6). The method was checked against the more specific method involving precipitation as the digitonide and no systematic difference was found. Cell cholesterol concentration was estimated directly by the same method on 0.2 ml aliquots, in duplicate, of a one-to-one dilution of washed cells with distilled water. The hematocrit value was obtained by centrifuging for 60 minutes at 1700 rpm (800 x g) in Wintrobe tubes. In all cases precautions were taken to prevent hemolysis and the serum was separated immediately after clotting.

Results. The standard error of 50 pairs of duplicate direct analyses of cell cholesterol was $\pm$ 2.4 mg/100 ml, calculated as $(S.E.M.)^2 = (\Sigma\Delta^2)/2N$, when N is number of pairs and Δ is difference between duplicates. This is of the same order as, but actually slightly smaller than, standard error of measurement found with the same method applied to serum(7).

Results on 38 casual blood samples are summarized in Table I. Indirect and direct estimates of cell cholesterol concentration gave excellent agreement between the means 137.6 and 137.7 mg/100 ml respectively. The variability of the value obtained indirectly was almost twice that of the direct estimate but even so was only a small fraction of the serum variability. Concentration of cholesterol in the cells was therefore relatively constant and was not correlated ($r = 0.07$) with serum concentration.

TABLE I. Total Cholesterol Concentration in mg/100 ml in Serum and in Cells in Blood Samples from 38 Men.

	Serum	Cells	
		Direct	Indirect
Mean	238.8	137.7	137.6
Stand. dev. (inter-individual)	48.8	6.5	10.5

Fig. 1 summarizes results from the dietary experiments. In the face of a mean serum change of –63.8, corresponding cell values, measured directly, showed a mean change of only –3.6 mg/100 ml. With a serum change of +51.8, the cell change averaged –2.0 mg/100 ml. These cell concentration changes are statistically not significant.

Discussion. The values for cell cholesterol reported by Foldes and Murphy(3,4) are higher and far more variable than our findings. The Bloor method they used gives values for plasma or serum averaging about 15% higher than the present method. This is explained by the fact that cholesterol esters give a more intense color with the Liebermann-Burchard reagent than does free cholesterol. Since pure free cholesterol is always used as the standard it is obvious that a method that does not hydrolyze the esters

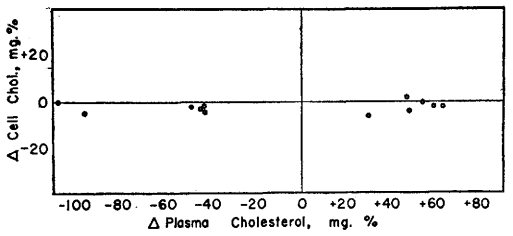

FIG. 1. Changes in plasma and in erythrocyte cholesterol concentration induced by dietary alterations. Each point represents change in blood concentration of an individual between 2 diets.

must give erroneously high values. But on detailed computation this factor does not seem to account fully for the discrepancy between our mean values and those of Foldes and Murphy though it could account for some of the greater variability of the latter data.

It is well known that all or almost all of the cholesterol in erythrocytes is in the free form(1,2,8) whereas except in a few disease states the ester form is a constant fraction of about 71% of total cholesterol in plasma or serum. There is a rapid exchange of labelled cholesterol between cells and plasma(9,10) but apparently the cells offer only a fixed number of acceptor sites for free cholesterol and admit no ester cholesterol. Since when cells are laked the cholesterol in them all appears in the stroma(11), it follows that the cholesterol acceptor sites in the cells are in that portion. It seems reasonable to suggest that any inter-individual variability in cell cholesterol concentration must be related to individual differences in the relative volume or surface of the stroma per unit volume of the erythrocytes. All of the present data suggest that the stroma per unit volume of erythrocytes must normally be very constant although a very small real variability between individuals is indicated.

Summary. 1) Direct analyses of cholesterol in blood cells from 38 men yielded average value of 137.7 mg/100 ml and there was no correlation with serum values in these samples. Standard deviation of these cell values was only 6.5 and part of this small value is accounted for by analytical error. Indirect estimates agreed closely but variability was larger. 2) In 2 dietary experiments on 6 men which produced average changes of −63.8 and +51.8 mg of cholesterol/100 ml of serum, there were no significant changes in cholesterol concentration in the cells.

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Selective Inhibitory and Teratogenic Effects of 2,5-Alkylbenzimidazole Homologues on Chick Embryonic Development.*† (25842)

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Many studies have shown that compounds differing widely in molecular structure, as well as more closely related compounds, elicit specific teratogenic responses(1-7). In addition, compounds as closely related as homologues also have this ability as demonstrated by Ambellan(8) with phosphonucleotides in amphibians. Homologous alkyl benzimidazoles had a characteristic order of inhibition. Hendlin and Soars(9) first reported that increasing length of alkyl chain in the 2-position from 0 to 4 carbons caused a corresponding increase in inhibitory activity in bacteria. Similar results were found in chicken sperm with alkyl chains of 1 to 7 carbons(10). However, Tamm *et al.*(11) found that lengthening the chain from 2 to 4 carbons did not cause further increase in inhibition of virus multi-