bottom of the dish they were shaken, at 105 rotations/min, on a variable-speed rotator (*9*) for periods of 2 seconds every 16 seconds. At first the animals contracted in response to each 2-second shaking period. As shaking continued, the proportion of animals that contracted decreased to an asymptote value (Fig. 2). Thus, *H. pirardi* becomes accustomed to intermittent nonlocalized mechanical stimulation.

Since *H. pirardi* was observed to contract in response to strong light and to become accustomed to mechanical agitation, an investigation was made to determine whether a light stimulus would still elicit the contraction response from animals habituated to shaking. In one experiment, five animals that had been starved for 24 hours were placed in 100 ml of water in a shallow dish of 300-ml capacity at 21°C. The dish was shaken at 50 rotations/min for 1-second periods every 19 seconds. The proportions of animals that contracted in response to shaking for 1 second were recorded (Fig. 3, top).

The contraction rate reached the asymptotic value after approximately 20 hours of shaking. When the animals had become accustomed to the shaking, the strong light was directed on them for 1-minute periods, while shaking was continued. In Fig. 3, bottom, the solid columns of the histogram show the proportion of animals that contracted during the 1-minute periods of light; the open columns indicate the proportion of animals that contracted spontaneously or in response to the shaking alone. In 20 trials of response to light, all the animals contracted on each exposure to light. Occasionally an animal contracted, expanded, and contracted again during the 1-minute period. This uniformly high rate of contraction in response to light demonstrates that *H. pirardi* contracts in response to this stimulus even though it no longer contracts on being shaken.

When this experiment was repeated with a lamp of much lower intensity (*10*) than the original light source, similar results were obtained. These results also show that the time between the onset of the light and the contraction response is inversely related to the intensity of the light. In addition, there is a wavelength specificity for the contraction of *H. pirardi* in response to light. The time between the onset of the light and the beginning of a contraction is shortest for blue light (400 to 450 mμ) (*11*). After these experiments had been completed we learned of the recent report of Passano and McCullough (*12*) that *H. pirardi* display rhythmically reoccurring electrical potentials. Their results show that blue light changes both the frequency and the point of origin of these potentials. Since the potentials are related to the contraction response, our behavioral observations support this finding.

It appears that *H. pirardi* does not become accustomed to a light stimulus but does become accustomed to mechanical stimulation. Indeed, in more recent experiments we found that the contraction rate was not reduced after 200 hours of intermittent exposure to light. We have also discovered that excision of all tentacles completely inhibits the contraction response to mechanical agitation. Such removal of tentacles does not affect the contraction response to light. However, removal of tentacles and hypostome inhibits the response to both stimuli (*11*). These results, together with those described earlier in this report (*13*) suggest that *H. pirardi* has different receptors for light and for mechanical agitation. In addition, the fact that an animal showing a reduced rate of contraction in response to mechanical stimulation still contracts in response to light suggests that the control mechanisms for the contraction responses to these two stimuli are independent of each other.

NORMAN B. RUSHFORTH
ALLISON L. BURNETT
RICHARD MAYNARD

Developmental Biology Center, Western Reserve University, Cleveland, Ohio

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Human Lipoproteins: Role in Transport of Thyroid Hormones

Abstract. *Lipoproteins from patients receiving therapeutic doses of iodine-131 were isolated by density-gradient techniques. The binding of circulating thyroid hormones by beta lipoproteins (those of low density) was negligible. Alpha lipoproteins (those of high density) bound appreciable amounts. The bulk, however, was bound by proteins of density higher than 1.23 g/ml.*

Although it is generally accepted that various circulating plasma proteins are capable of transporting thyroid hormones, there is no general agreement about the nature of the linkage of thyroxine and triiodothyronine to these (*1*). The different methods used have yielded somewhat different results (*2*). The thyroid hormones appear to be bound mainly to an alpha globulin migrating between the alpha-1 and alpha-2 globulins (thyroxine-binding globulin, TBG) when the human serum containing physiologic concentrations of thyroid hormones is subjected to paper electrophoresis in barbital buffer (*p*H 8.6), but no detailed analysis of the chemical nature of thyroxine-binding globulin has been performed. There is evidence that TBG may be a glycoprotein (*3*).

J. R. Tata has indicated that there are some lipids in addition to carbohydrates in isolated TBG (*4*). Clausen and Munkner, using a combination of immunoelectrophoresis of human serum incubated with radiothyroxine and radiotriiodothyronine and autoradiographic techniques, concluded that the only proteins in human serum which bind hormones are the three known lipoproteins, the alpha-2 lipoproteins (the slow moving lipoprotein fraction), alpha-1 lipoprotein (the faster moving lipoprotein), and lipalbumin (*5*).

If this is so, separation of lipoproteins from sera which have been incubated with radiothyroxine or triiodothyronine or removal of lipoproteins from sera of patients who have received doses of I^{131} would contain practically all the radioactivity bound in organic materials.

This separation can be accomplished without too much difficulty by density gradient techniques by taking advantage of the difference in density between the proteins containing lipids and the remaining proteins (*6*). Of course, these lipoproteins must be isolated without too much handling to obtain them

in a state very close to the native one.

Blood samples from seven patients who had received doses varying from 2 to 6 mc of I^{131} for hyperthyroidism were collected 24 and 48 hours after the dose and allowed to clot. The serum removed after centrifugation was treated with an anionic exchange resin (Amberlite IRA-400 in its chloride phase) to remove the inorganic I^{131} and was then brought to a density of 1.063 g/ml with dry sodium bromide (*7*). All densities were measured at 25°C with a 1-ml pycnometer. Enough serum was used to fill a 13.5-ml Lusteroid centrifuge tube. The tube was centrifuged for 18 hours at 105,000*g*. At the end of this period a density gradient was found; the minimum density (at the top) was 1.050 g/ml, and the maximum density (at the bottom) was 1.076 g/ml. The tube was cut into 10 equal portions. The volume of each portion was measured, and its activity was determined with a well-type scintillation counter.

The beta lipoproteins (low-density lipoproteins), with density varying from .980 to 1.040 g/ml, appeared at the top as a very yellow layer, while all other serum proteins sedimented toward the bottom of the tube. Only the first fraction contained beta lipoproteins (*8*). The layer next to the first contained negligible radioactivity. The contents of the remaining nine portions were pooled, the volume was brought to 13 ml with saline solution and the density was brought to 1.216 with dry sodium bromide.

This solution was centrifuged for 36 hours and then cut into ten approximately equal portions. A density gradient was found, with a minimum density at the top (1.190 g/ml) and a maximum at the bottom (1.248 g/ml). The high-density lipoproteins appeared at the top of this layer as a very yellow band (*9*). Once again, the layer next to the first contained negligible radioactivity. The high-density proteins (1.220 g/ml or higher) sedimented toward the bottom.

Our results in eight experiments (*10*) showed that under the above-mentioned experimental conditions the low-density lipoproteins (or slow-moving lipoproteins in electrophoresis at *p*H 8.6) bind very little of the circulating thyroid hormone. The range varied from 0.5 to 2.0 percent of the total radioactivity. The binding of thyroid hormones by the high-density lipoproteins (or fast-moving lipoproteins in electrophoresis at *p*H 8.6) was appreciably higher, being from 8 to 12 percent of the total radioactivity. The bulk of the radioactivity was bound to proteins of density higher than 1.23 g/ml; these proteins obviously are not lipoproteins. The possibility exists that the binding of thyroid hormones by proteins may be affected by high concentrations of salt. If this were so, it could explain the difference between our results and those of Clausen and Munkner (*5*). Although experiments performed in our laboratory show that high salt concentrations do not diminish the capacity of serum to bind thyroid hormones, alterations in the binding by lipoproteins caused by high concentrations of salt, if any, are not known. This may merit further study (*11*).

E. TORO-GOYCO
MARTA CANCIO
Veterans Administration Hospital and
Department of Biochemistry and Nutrition, School of Medicine, University of Puerto Rico, San Juan

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8. We verified that no other protein was present in this fraction. This was determined by paper electrophoresis in barbital buffer at *p*H 8.6, the strips being stained both for protein with bromphenol blue and for lipid with oil red O.
9. The presence of only lipoproteins in this fraction was also determined by electrophoresis in barbital buffer at *p*H 8.6 and staining of the paper strips both for protein and lipids.
10. The eighth was an additional experiment performed in which serum from a normal patient was tagged in vitro with commercial radioactive thyroxine. The results did not differ from those obtained in the other seven.
11. Support by the National Institutes of Health (research grant A-4251) is acknowledged.

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Crystal Structures at High Pressures of Metallic Modifications of Silicon and Germanium

Abstract. *Studies of germanium and silicon by x-ray diffraction reveal that their crystal structure changes at high pressures from the semiconducting diamond-type structure to the metallic white tin structure, in analogy to the known "gray" to "white" transition in tin itself.*

The development of a new x-ray diffraction technique (*1*) has made it possible to obtain useful information about the crystal structure of materials while they are exposed to pressures above 100 kilobars. I have obtained results with silicon, germanium, indium antimonide, indium arsenide, gallium antimonide, aluminum antimonide, indium phosphide, and tin (*2*). The common-structure Sn-type transitions in these elements and compounds indicates that there is a systematic variation in the pressures at which the group IV elements and certain group III–V compounds attain the metallic state. I now report work on silicon and germanium (*3*).

The x-ray diffraction cameras operate on the same physical principle as that described previously (*1*). However, to improve the quality of the picture and the precision, a camera diameter of 114.6 mm is now used in conjunction with commercial collimators (*4*). The film holder is fixed in position with respect to the sample, and diffraction lines may be obtained at 2θ values from 10° to 170°. But, since the sample is encased in "amorphous" boron, which cuts down on the diffracted line intensity, and since filtered Mo radiation is used, the high pressure patterns seldom have useful lines above $2\theta°$ equals 60°.

An annulus of "amorphous" boron