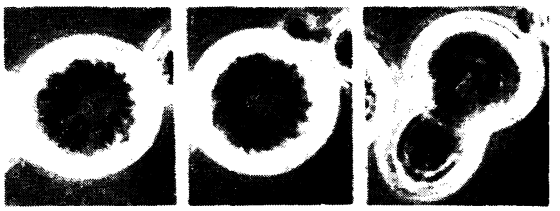

FIG. 2. Three views of a neuroblast cell in anaphase in a typical embryonic cell suspension prepared from a trypsin-hyaluronidase digest in calcium-free salt solution. Rotation of the cell has occurred so that two of the photomicrographs are polar views and the third is a side view. Neuroblasts normally divide unequally: the smaller daughter cell is the ganglion cell. Other cell types in the suspension divide equally.

it is not possible to determine whether the low value of cell respiration in the suspension is due to loss of cells in the separation, or to a decreased rate of respiration per cell.

For 7–8 hr after separation, dividing cells can be seen in suspension (Fig. 2). Detailed observations on division have been limited to mid-mitotic phases, and these phases proceed as in the intact embryo, but cursory examination of other phases has revealed no deviation from the normal. Second divisions of neuroblasts in the suspension have been observed.

It should be feasible to isolate neuroblasts or other types of embryonic cells from such a suspension for particular cytological or physiological study. Although the use of this technique has been limited to grasshopper embryos, it should be adaptable to other embryonic as well as adult tissues where a cell suspension or isolation is desirable.

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Factors Influencing the Uptake of Iron by Blood and by Bone Marrow Cells *in Vitro*

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To investigate the mode of action of copper in iron metabolism, the technique used by Walsh *et al.* (*1*) to determine iron uptake by cells was used. The iron used was a mixture of Fe^{55} and Fe^{59} [2] and had a specific activity of approximately 2 mc/g Fe. It was added to the culture medium as ferrous ammonium sulfate. The blood cells were obtained from rabbits that were bled repeatedly to increase the reticulocyte count. The bone marrow cells were obtained from chicks or turkey poults.

The amount of iron uptake is dependent upon the washing of the cells in 0.93% NaCl prior to incubation. One milliliter of heparinized blood (reticulocyte count 6%, hemoglobin 9.5%) gave a count of 150 cpm after 8 hr of incubation. When washed once in 3 volumes of saline, before incubation, a count of 640 cpm was obtained. The 100 μg of Fe used gave a count of 25,700 cpm. In another experiment (11.7% reticulocytes) the cells were washed one, two, and three times and counts of 540, 900, and 930 cpm, respectively, were found. The influence of copper, the number of cells, and the iron level are shown by the data in Table 1.

TABLE 1.

Number of cells (washed once)	100 μg Fe 9 μg Cu (cpm)	100 μg Fe (cpm)	50 μg Fe (cpm)
1.63×10^6	1770	460	440
3.26×10^6	950	650	460
6.52×10^6	880	620	610

The addition of copper increases the iron uptake, particularly with fewer cells. Reducing the amount of iron has little effect. Where copper is present, fewer cells take up more iron than do larger numbers. In another trial, halving the iron approximately halved the uptake.

Similar results were found with bone marrow cells. These were obtained by crushing the long bones in isotonic saline, filtering through glass wool to remove extraneous material, and further isolation by slow centrifugation, following the technique of Magnussen (*2*). When resuspended in saline and again centrifuged, the washed cells took up 2.4 times more iron than those not washed. A second washing caused no further uptake. The effect of iron and copper levels is shown in Table 2.

It can be seen that as the level of iron supply is increased, the amount of iron uptake is increased. In-

TABLE 2.

μg Fe added	μg Cu added as the acetate (cpm)			
	0	5.7	11.4	22.8
10	270	350	440	870
40	1300	1800	1800	3000
120	4700	7400	7400	8300
120	3600*			200*

* Incubated 20 min; others, 6 hr. Total cells/culture tube 1.25×10^6: 100 μg Fe gives 12,700 cpm. Each datum is the average of 2 runs. Cells were from 5- to 6-wk-old chicks.

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creasing the copper supply does the same, particularly at the lower level of iron. The uptake of iron is greatly reduced as the time of incubation is shortened.

Walsh *et al.* (*1*) reported a correlation between reticulocyte count and iron uptake when using blood from pernicious anemia patients in remission, and concluded that their results could be interpreted in physiologic terms. In our studies with rabbit blood this correlation was not obtained. The iron uptake by blood cells was considerable and was increased by the inclusion of copper in the culture medium. As the amount was influenced by several other factors and was far in excess of that which could possibly be used in hemoglobin synthesis, this result is not interpreted in physiologic terms. Our results indicate that the mechanism which determines iron uptake must be fairly complex.

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Radioactive Gold in Filter Paper Electrophoresis Patterns of Plasma

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When radioactive colloidal gold is injected into the pleural or peritoneal cavities of patients with effusions, small but measurable amounts of radioactivity circulate in the blood. Andrews *et al.* (*1*) have indicated that less than 1% of the given dose is in the blood at one time. This finding was confirmed in a series of patients treated at the Mount Sinai Hospital (*2*). Less than 0.2% of the given dose in the serous cavity was encountered in the circulating blood.

The gold in the blood is in the plasma; the red blood cells and white blood cells contain no measurable radioactivity. When trichloracetic acid is added to plasma the gold is in the protein precipitate. To determine whether gold in plasma is coprecipitated with or bound to protein presents a problem. If the gold is bound to protein it is of further interest to know which protein fractions bind the gold.

Attempts to solve these problems by using chemical methods of protein separation have yielded inaccurate results. Separating the proteins by electrophoresis would be a cumbersome procedure. Filter paper electrophoresis, however, is a method that simplifies the separation of plasma proteins (*3*) and lends itself to the determination of which protein fractions bind radioactive gold (*4*). In this method a current is passed through a strip of filter paper which has been soaked in buffer solution. The ends of the filter paper dip into positive and negative electrode vessels. Samples of protein-containing solution are applied to the paper, and proteins migrate with the current. Separation of the protein fractions is satisfactory in a few hours. These fractions can be dyed differentially for identification.

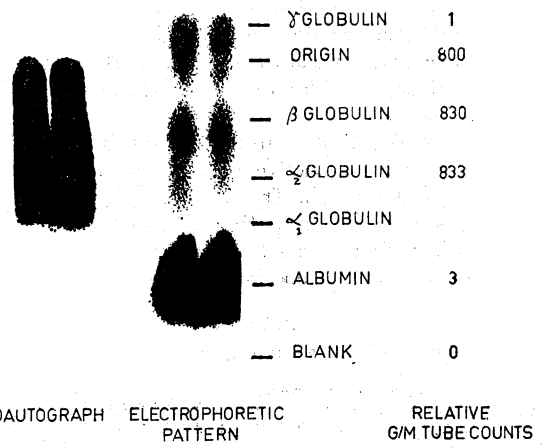

FIG. 1.

Radioactive colloidal gold (Au^{198}) was added to normal plasma *in vitro* (500 μc Au^{198} to 200 cc plasma), and samples of this solution were precipitated with trichloracetic acid at time intervals following the mixture of these two components. Counting the radioactivity of these precipitated samples showed that the uptake of radioactive gold in the proteins was maximal within 15 min. The proteins in the original solution containing gold (before precipitation) were then separated by applying about .030 cc of the plasma to filter paper. Figure 1 shows the filter paper electrophoretic pattern of these separated proteins and identifies the fractions. The distribution of radioactive gold in this pattern was determined by Geiger-Muller tube counts of various areas in the electrophoretic pattern as indicated in Fig. 1. The thin window (5 mg/cm^2) of the tube was 6 mm in diameter. A radioautograph of the same electrophoretic pattern confirmed the binding of radioactive colloidal gold to the α and β globulin fractions of the plasma proteins; no gold was bound to the γ globulin or albumin. As indicated in Fig. 1, considerable radioactivity remained at the site of the originally applied droplet of plasma on the filter paper.

This process was repeated with plasma from a patient who had received an intrapleural injection of 60 mc of Au^{198} 24 hr previously. The distribution of Au^{198} in the plasma protein in this patient is similar to the distribution in the plasma to which gold had been added *in vitro*.

In the filter paper electrophoretic pattern of ascitic fluid from a patient who had carcinoma of the pancreas, the ascitic fluid was aspirated from the peritoneal cavity one day after the intraperitoneal injection of 142 mc of Au^{198}. The gold is distributed in essentially the same fractions as in the blood. Some of the gold is unfixed to the globulin and remains at the point of application (origin) of the ascitic fluid on the filter paper.

[1] The author acknowledges the cooperation of Arthur W. Ludwig in this work.