could not have significantly affected the osmotic value of the nutrient, and cell division was obviously not suppressed. Another possibility, suggested by Newcomb (*3*), that lack of ascorbic acid might be important for the breakdown of the middle lamella, is likewise not a sufficient explanation here, for other cultures of normal tissue on nutrients with widely varied concentrations of vitamin C (10^{-7} to 10^{-4} g/ml), and even without this compound, formed hard, crustose calluses.

It seems probable that three factors may be involved in the transformation of spruce cultures: (i) some as yet undefined peculiarity in certain tissues; (ii) a change in the physiological characteristics (probably in the protein and carbohydrate metabolism) of the dissociating conifer tissues; and (iii) a selection of particularly fast-growing cells during the long period (December 1953 to August 1954) during which the cultures were maintained on a nutrient containing a growth-restricting component (folic acid). These possibilities will have to be explored. Whatever the explanation, the possibility of establishing at will large numbers of cultures derived from single cells or small groups of cells can be important for an analysis of various cellular changes.

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9 September 1955

Experimental Monocytic Leukemia

It is now generally recognized that the essence of leukemia consists in the inability of the immature leucocyte to respond to the forces that normally regulate its maturation and proliferation. This occurs because the matrix of the specific tissue produces pathological leucocytes. But we know neither the factors that regulate the production, nor those that regulate the maturation of the white cells in the places of origin, nor, finally, those that regulate the level of blood leucocytes, under normal and pathological conditions.

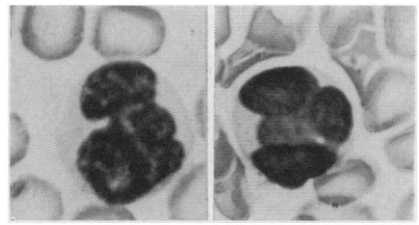

Fig. 1. Rabbit blood: two pathological cells.

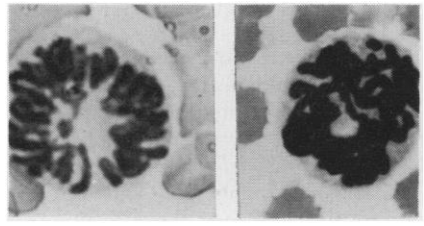

Fig. 2. Rabbit blood: two atypical mitoses.

Researches on the pathogenesis of human leukemia must obtain these results: (i) proliferation of the reticuloendothelial system in a specific way, with production of pathological leucocytes; (ii) immaturity in these leucocytes; and (iii) passage of these cells into the circulating blood, demonstrating that they no longer respond to the forces that regulate their maturation and proliferation. These results were obtained in the experiments reported here. Rabbits of average weight 2 kg were repeatedly injected with different proteins. The most efficacious proved to be lactoglobulin, lactoalbumin, egg albumin, and several others. Whole cow's milk, inoculated as soon as it had been milked, always gave excellent results. The blood alterations relate principally to leucopoiesis, whereas the erythropoiesis is altered only in a terminal stage, when the animal reaches a stage of cachexia.

The increase in the number of leucocytes is considerable. Instead of 8000/mm^3, as is average for the normal rabbit, the number of 30,000 to 40,000 is reached after a prolonged treatment, for example, with milk or with lactoglobulins. The smears of blood indicated leukemia. Monocytes and monoblasts predominated, appearing often with a monstrous nucleus and giving the cells a neoplastic aspect (Fig. 1). Of great importance was the presence of numerous cells with direct and indirect division, either bipolar or multipolar, typical or atypical, symmetrical or asymmetrical (Fig. 2). The most important alterations were observed in the spleen (Fig. 3), in the liver (Fig. 4), in the bone marrow, and in the lymph nodes; but thymus, kidneys, adrenals, and lungs were also involved.

The principal alterations occurred in the reticuloendothelial system. In fact, the reticuloendothelial cells of the sinusoids of the lymph nodes, of the spleen, and of the liver, and the living cells of the capillaries of the bone marrow and of the macrophages all are influenced by the treatment. From these cells originate cells of the monocytic type and their pathological forms just described, which multiply luxuriantly in the organs and in the blood.

The findings are those characteristic of human leukemia: (i) the production of pathological leucocytes in the organs of animals treated with heterologous proteins, in which an extended metaplasia is found, especially in the spleen, liver, bone marrow, kidneys, lymph nodes, and wherever active mesenchymal tissue is present; (ii) the permanent increase of white cells, especially of pathological type, highly immature, and sometimes abnormal. These elements continue to multiply through a direct division and through mitosis in the circulation. Therefore, these cells do not respond to the factors that under normal conditions regulate their maturation and their proliferation.

On the basis of my experiments, I can conclude that the heterologous proteins influence the monocytic potentiality of

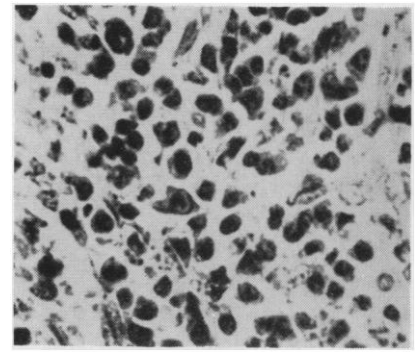

Fig. 3. Spleen of rabbit treated with lactoalbumin.

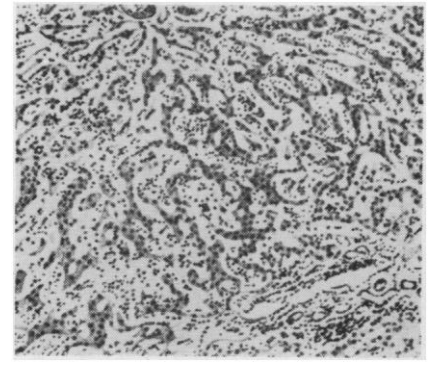

Fig. 4. Liver of rabbit treated with whole cow's milk.

the reticuloendothelial system without participation of the other potentialities of the system, yielding a unique monocytic cell. Both extramedullary and medullary proliferation concerns exclusively this type of cell. The experimental condition produced by me corresponds to the form known in human pathology as Schilling's monocytic leukemia. This germinative line of leucocytes has therefore in pathology its meaning and importance, as Virchow claimed.

These experimental results may be considered in the light of what we know with respect to the origin of certain neoplasms, so-called "conditioned" that are best known in the field of endocrinology. Leukemogenesis is a type of carcinogenesis (Furth) and therefore the concepts referred to the growth of conditioned neoplasms can be extended to experimentally ascertained events that move in the direction of leukemia.

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Copper Fluorides

A recent paper of Crabtree, Lees, and Little (*1*) reports in part on an x-ray study of the fluorides of copper. Reference is made to an earlier paper by Ebert and Woitinek (*2*), the conclusions of which are questioned. In attempting to explain the original error, the recent authors have confused the situation by further error and misinterpretation.

The complete explanation had previously been published (*3*). In brief, the original "CuF," $a = 4.26$ A, ZnS structure, was apparently Cu_2O, $a = 4.25$ A, Cu_2O structure. "CuF_2," $a = 5.41$ A, CaF_2 structure, was apparently CuCl, $a = 5.41$ A, ZnS structure. The comparison becomes obvious when the interplanar spacings are calculated, taking into account the extinctions characteristic of the different structural types. Direct comparison of lattice constants is not advisable since the structure types being compared are not the same, contrary to the statement by Crabtree *et al.* (*1*). The general extinctions of space groups $F\bar{4}3m$ (CuCl) and $Fm3m$ ("CuF_2") are the same, but those of $F\bar{4}3m$ ("CuF") and $Pn3m$ (Cu_2O) are not.

To complicate matters further, reference is made to cupric chloride, $CuCl_2$, with lattice dimension 5.4075 kx, where CuCl, $a = 5.4057$ A (*4*) is meant. Cupric chloride has the monoclinic $CuBr_2$ structure.

The structure of cupric fluoride, CuF_2, has been determined here by Claudine Billy. It is monoclinic, $a = 3.325 \pm 0.005$ A, $b = 4.544 \pm 0.005$ A, $c = 4.578 \pm 0.005$ A, $\beta = 83°17'$. Details will be submitted elsewhere for publication.

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19 September 1955

Paleotemperatures of Pacific Bottom Waters and Multiple Hypotheses

Cesare Emiliani's recent article on the temperature decrease of Pacific bottom waters during the Tertiary (*1*) is not only a very interesting and important contribution but one that stimulates many lines of thought. Using the method of oxygen isotope analysis, he has analyzed the benthonic Foraminifera of three cores from the eastern Pacific, which he reports as Oligocene, Miocene, and late Pliocene, indicating a temperature decrease of about 8°C from the middle of the Oligocene to the end of the Pliocene. Emiliani's conclusions are predicated on at least two critical assumptions that permit additional postulates. He assumed, in the first case, that one or two species dated the entire fauna in *Albatross* cores 53 and 57 and, in the second case, that the entire fauna analyzed lived in the same abyssal depths as the one or two key species.

Concerning the first assumption regarding age data, Emiliani states that the Middle Oligocene age of core number 53 is well established because of the presence of typical specimens of *Cassidulina spinifera* Cushman and Jarvis in all of the samples of the core. He reports that Oligocene and Miocene sediments were available for coring in cores 53 and 57 because submarine erosion or slumping had previously eliminated the younger sediments. It should be pointed out that *Cassidulina spinifera* or one almost identical with it occurs in association with modern species in the Marshall Islands at depths of 710 and 680 fathoms (*2*). There are many typical specimens of this species in the collections of the Allan Hancock Foundation, all from Recent assemblages collected in the Marshall Islands. Other reports indicate that this species is no older than Pliocene in the Pacific area (*3*). Cushman and Stainforth also recognize discrepancies in the correlations between Trinidad and the Pacific area. In a report on *Cassidulina spinifera* and the associated microfauna of the type locality for this species (Oligocene, Cipero formation, Trinidad), they speak of the striking similarities between the Cipero species and those of the late Tertiary of the Indo-Pacific region. They also mention T. W. Vaughan's comments regarding the affinities between the Oligocene corals of Trinidad and the living species of the Indo-Pacific area (*4*).

Next, Emiliani states that the age of core 57 is established as lower-middle Miocene because of the presence of *Gyroidina zelandica* Finlay, together with *Laticarinina bullbrooki* Cushman and Todd. This species of *Gyroidina* is very similar to variations of *G. soldanii* that range from well back in the Tertiary to Recent. As for *Laticarinina bullbrooki,* the very authors of this species state, "A very similar, perhaps identical, species occurs in some numbers at *Albatross* D 2144 at 896 fathoms in the Caribbean Sea off Panama" (*5*).

Arrhenius (*6*) analyzed core 58 by the titanium method of age determination, and the Pliocene sample in Emiliani's report was taken from just below the probable Pleistocene core segment thereof.

Turning to the second assumption, that the faunas are abyssal, again raises a question. One might postulate that the same results might be achieved with displacement of shallow water faunas instead of decreasing bottom temperatures. If the faunas were displaced from depths of about 500 m or less, the temperatures there are mostly above 7°C, and so isotopic analyses would produce results that would be within the range reported by Emiliani for the Oligocene and Miocene cores. True, the key species include two that are most likely abyssal in habit (*Laticarinina bullbrooki* and *Gyroidina zelandica*); however, the remaining faunas in the two cores may include shallow benthonic species, and these would in turn affect the results. *Cassidulina spinifera* occurs in the Oligocene of Trinidad with an upper bathyal fauna, and it also occurs in modern sediments on the flanks of some of the seamounts of the Pacific along with modern species. Whether or not this species is fossil and/or displaced, it is inconclusive evidence that the associated species in the isotope analyses are Oligocene or abyssal.

On the basis of the published report, there is a reasonable doubt concerning the ages of samples 53 and 57 and there is similarly little reason for indicating ages of faunas based on one or two species of faunas that are taken from the surface sediments of the sea bottom. An excellent example of possible pitfalls of this kind is demonstrated by Hamilton in his paper on the mid-Pacific seamounts (*7*). He reports mixtures of Cretaceous to Recent species in a sample taken at 2050 fathoms at latitude 19°34'N, longitude 171°54'W near the