

No undesirable aftereffects have been observed in animals given overdoses of the drug.

Several members of the class were tested for acute toxicity in rodents and dogs by oral and parenteral routes. Among these, 3-methyl-pentyne-ol-3 was notably low in toxicity. The acute oral LD₅₀ for mice, rats, and guinea pigs, ranged from 600 to 900 mg/kg. The animals died in a state of coma. A few active compounds with low acute toxicity were tested for chronic toxicity in mice, rats, and dogs. 3-Methyl-pentyne-ol-3 at 200–300 mg/kg/day (approximately 70 times the recommended human dose) did not produce any gross or micropathological changes. Blood sugar levels, hemoglobin values, and erythrocyte, white blood cell, and differential counts were normal. Dogs that had received chronically 3-methyl-pentyne-ol-3 *per os* showed normal renal function (phenolsulfonphthalein test).

In metabolic studies on 3-methyl-pentyne-ol-3, the acetylenic hydrogen was used to identify and estimate the compound through the formation of the silver acetylide and the microdetermination of silver (9). Following the oral administration of 3-methyl-pentyne-ol-3 (200 mg/kg) to adult dogs, 0.5–4.6% of the total dose was excreted in the urine during the first 24 hr; thereafter, only traces could be detected. In preliminary trials, no drug was detected in the urine obtained from three human subjects during a 60-hr period after a single oral dose of 100 mg. It was estimated that no more than 8% of the total dose could have been present in any 4-hr specimen. Following intravenous administration to dogs (200 mg/kg), it was found that the blood contained during the first 10 min 20% of the total dose; no drug could be detected in the blood at 2 hr. Analyses of rat tissues (brain, spleen, kidney, adipose, muscle, and liver) taken while the animals were still under the hypnotic action of 3-methyl-pentyne-ol-3 (800 mg/kg), revealed that adipose, muscle, and liver tissues together contained approximately 20% of the total dose. No unchanged compound was found in any of the organs or tissues when the effects of the drug were no longer manifest. *In vitro* experiments in which 3-methyl-pentyne-ol-3 was added to whole blood from dogs and rats indicated that there was no breakdown of the compound; i.e., the acetylenic hydrogen was still present. However, slices of kidney or liver, and to a lesser degree slices of brain, changed this molecule in such a way as to render it nonreactive with the silver reagent (9).

In a clinical study in 134 subjects, the majority of whom previously required barbiturates for sleep, 3-methyl-pentyne-ol-3 was found to be highly active, without toxic effect, and free from undesirable side actions (10). The effective oral dose in adults was 200–300 mg. Sleep was brought about in the majority of patients in less than ½ hr. The patients who received 3-methyl-pentyne-ol-3 experienced restful sleep and had no "hangover" upon awakening. A number of patients have been given daily doses of the compound for more than 6 months without any untoward effects.

Complete blood counts, urinalyses, blood sugar, blood urea nitrogen, creatinine, total serum protein, albumin, globulin, phosphorous, alkaline phosphatase, total cholesterol, free and combined cholesterol, and in addition, the icterus index, Van den Bergh, thymol turbidity, or cephalin flocculation values were determined, before and after medication with 3-methyl-pentyne-ol-3. These clinical laboratory tests indicated that there were no pathological changes attributable to the drug.

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The Life Span of the Red Blood Cell in Chronic Leukemia and Polycythemia^{1,2}

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The anemia associated with leukemia and neoplastic diseases is still not completely understood; infiltration of the bone marrow in some cases, with crowding out of the erythropoietic tissue, has been postulated as a significant factor in the pathogenesis of the anemia. However, Huff and co-workers (1) have shown with radioactive iron that in some patients with leukemia the rate of production of red blood cells was greater than the 0.8% per day that would be anticipated if the cells lived a normal life span of approximately 120 days (2). These same workers showed that in polycythemia vera the rate of production of red cells was much greater than 0.8% per day. These findings may be explained by postulating a decrease in the life span of the red blood cells in these diseases. Using N¹⁵-labeled glycine, which Shemin and Rittenberg (2) demonstrated to be a specific precursor of the porphyrin of hemoglobin, London *et al.* (3) found a normal life span of the red blood cell in a single case of polycythemia vera.

Since C¹⁴-labeled glycine has been shown to be satisfactory for the labeling of rat hemoglobin (4, 5) and for the determination of the life span of the red blood

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cell of the dog (5, 6) and rat (7), studies (8) utilizing C^{14} -methyl-labeled glycine were undertaken to determine the red blood cell life span in patients with leukemia and polycythemia.

Three patients with chronic leukemia, one with lymphatic and two with myelogenous, and two patients with polycythemia vera were given intravenously 8.6 mg of glycine $2-C^{14}$ containing 100 μ c of C^{14} . Blood samples were drawn at frequent intervals, and the hemoglobin was extracted by a modification of the method of Drabkin (9). The plasma was removed by centrifugation, the cells were washed three times with saline and then lysed with distilled water; the lipids were extracted with toluene. After standing overnight, the solution was centrifuged and an aliquot of the clear hemoglobin solution was dried *in vacuo* and combusted to CO_2 by the method of Van Slyke and Folch (10). This was precipitated as $BaCO_3$; a 1-g aliquot was converted to CO_2 and the specific radioactivity measured in a 100-ml ionization chamber (11), using a vibrating reed electrometer and recording potentiometer.

Figs. 1 and 2 show the graph for the specific activity of hemoglobin as a function of time in the three patients with leukemia (Fig. 1) and one patient with polycythemia vera (Fig. 2).

Fig. 1 shows that the red blood cells in one patient with chronic lymphatic leukemia in clinical and hematological remission at the time of these studies had a normal mean life span (110 days), and that in the two patients with myelogenous leukemia who were anemic there is considerable shortening of the mean life span of the red blood cell, from a normal of approximately 120, to 71 and 76 days, respectively. If the bone marrow in these patients continued to produce a normal number of red blood cells per day, and if these lived only 70 instead of 120 days, it is then evident that these patients will become anemic. Therefore, in leukemia, a significant factor in the pathogenesis of the anemia is the decrease in the life span of the red blood cell.

Fig. 2, which shows the specific activity of the hemoglobin as a function of time in one of the patients and is typical of both patients with polycy-

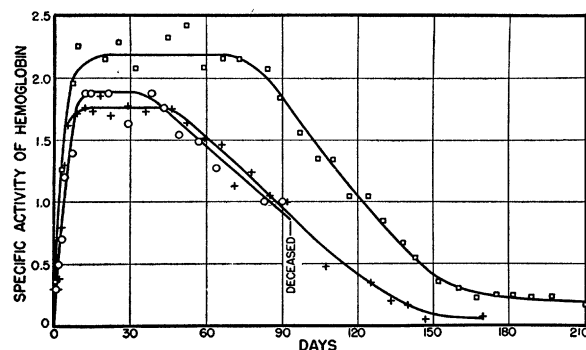


FIG. 1. The specific activity of the hemoglobin in disintegration/min/mg $BaCO_3$ as a function of time. Three patients with chronic leukemia. $\square$ = Chronic lymphatic leukemia; $+$, $\circ$ = chronic myelogenous leukemia.

themia vera, may be analyzed in the following manner: The graph from a period of 40–200 days is qualitatively similar to that of the normal and to the leukemic, so that in these individuals there is at least one class of red blood cells that had an almost normal life span; the rapid initial rise and fall in the first 15 days may be analyzed by considering this as due to the delivery into the peripheral circulation of a second class of red blood cells with a life span of only

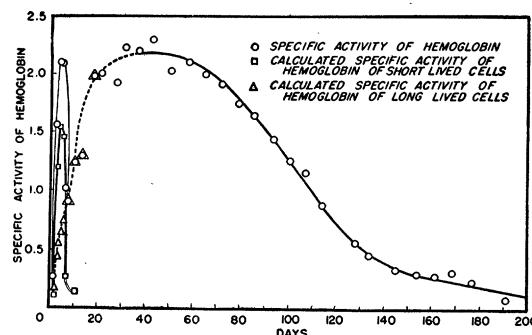


FIG. 2. The specific activity of the hemoglobin in disintegration/min/mg $BaCO_3$ as a function of time in a patient with polycythemia vera (Patient II. M.).

a few days. If the long-lived cells are delivered into the peripheral circulation at a rate consistent with a first-order process, which is what Shemin and Rittenberg (2) found, then, by determining the rate graphically in the interval 20–40 days after administration of the radioactive glycine, the curve for the specific activity of this class of cell may be extrapolated to the origin. The difference between the extrapolated curve for the long-lived cells and the observed specific activity is due to the presence of the short-lived cells, and so the curve for the short-lived cells may be constructed. It is assumed in the above discussion that in both classes of red blood cells the hemoglobin within the cell is not exchangeable and that the removal of the hemoglobin and the red blood cell from the circulation is the same process.

Huff and his co-workers (1), in their radioactive iron studies, have presented evidence which "may indicate that the longevity of red cells in polycythemia vera is significantly shortened." The present results show that such short-lived cells do exist, but that there is also present a class of cells with a normal life span. It is the rapid turnover of these short-lived red blood cells that is largely responsible for the greatly increased rate of utilization of iron for the formation of hemoglobin in this disease.

Further studies involving particularly the separation of the hemoglobin into the constituent porphyrin and globin moieties are in progress.

These studies may necessitate a revision of our present concept of the mechanism of the anemia occurring in neoplastic disease.³

³ Since the submission of this manuscript three additional anemic patients with chronic lymphatic or myelogenous leukemia have been studied and found to have red cells with decreased life span.

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Tissue Cytochrome *c* and Prevention of Experimental Atherosclerosis

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It has been shown that the protective action of iodides on the experimental atherosclerosis induced in rabbits by cholesterol-rich diets is exerted through the thyroid, since iodides are not effective in the absence of the gland (1, 2) or when given simultaneously with thiourea (2).

Drabkin (3) has pointed out that there is a clear positive correlation between the thyroid activity and the content of cytochrome *c* in the tissues. Consequently it seemed important to investigate whether changes will occur in the cytochrome *c* content of tissues of rabbits on a cholesterol-rich diet with potassium iodide, in the presence and in the absence of thyroid gland.

Rabbits on a cholesterol-rich diet (nearly 0.5 g cholesterol, in the form of cattle spinal cord, daily) and with constriction of the upper abdominal aorta inducing hypertension, which acts synergistically with diet in producing atherosclerosis (4), were divided into 4 groups: one control, another with thyroidectomy, and two on protective doses of potassium iodide (0.3 g orally every other day), one of them thyroidectomized. The animals were sacrificed 120 days after starting the diet. The development of atherosclerosis was judged macroscopically and evaluated on a scale of 0 to 10 (4). The cytochrome *c* of liver and kidney was extracted by the method of Potter and Du Bois (5) and determined spectrophotometrically according to Rosenthal and Drabkin (6).

The results on liver cytochrome *c* are given in Fig. 1. The changes in kidney cytochrome *c* were similar to those in the liver.

The correlation coefficient between liver cytochrome *c* and development of atherosclerosis is $r = -0.533$, with $t = 2.88$, a value regarded as statistically significant.

These findings confirm, in the rabbit, Drabkin's results in the rat (3) of the influence of the thyroid

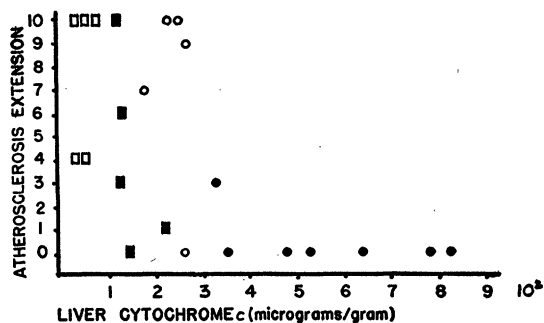


FIG. 1. Relationship between liver cytochrome *c* and degree of atherosclerosis in rabbits on cholesterol-rich diet, sacrificed at 120 days. Squares, thyroidectomized; circles, normal; open squares and circles, animals without treatment; solid squares and circles, animals given potassium iodide.

gland and thyroxine upon the level and content of cytochrome *c* in tissues. It is deduced from the data that the action of iodide may be one of stimulation of the thyroid gland, since the concentration of cellular cytochrome *c* is increased when the drug is administered to animals with thyroid. The results furthermore suggest that the augmentation of cellular cytochrome *c* must be considered as a factor in the prevention of the experimental atherosclerosis by means of potassium iodide.

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A Selective Medium for the Isolation of *Coccidioides immitis*

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Cultural procedures commonly used in attempting isolation of *Coccidioides immitis* from clinical specimens or from the physical environment frequently fail because of overgrowth of the pathogen by saprophytic fungi and bacteria. Laboratory tests indicate that a newly developed medium shows great promise in overcoming this difficulty.

Sabouraud dextrose agar fortified with penicillin (20 units/ml) and streptomycin (40 units/ml) was selected as a basal medium. This combination is inhibitory to most bacteria but does not prevent the growth of fungi, except the actinomycetes.

On the basis of the findings of Leach, Ford, and Whiffen (1), Whiffen (2), and Phillips and Hanel (3), which demonstrated the selective antifungal ac-