0.3%. It is obvious that the treatment has remained ineffective, and that at doses which allow about 50% of the ♂♂ and about 75% of the ♀♀ to survive, formaldehyde neither reacts with the chromosomes directly to produce mutations nor with the cytoplasm of the germ cells to produce a mutagenic substance.

TABLE 1

THE EFFECT OF FORMALDEHYDE VAPOR ON MUTATION RATE IN GERM CELLS OF IMAGINES

Sex	Survival rate	No. of tested X-chromosomes	No. of lethals	Percent of lethals
♂♂	43/60	1172	1	
	7/24	451	0	
	13/28	983	3	
		2606	4	0.2
♀♀	22/28	1136	0	
	21/28	1342	1	
		2478	1	0.05

Since it seems legitimate to consider the experiments as tests for spontaneous lethals, an interesting incidental result consists in the confirmation of two previously reported features of the spontaneous mutation rate. First, the mutation rate is higher in ♂♂ than in ♀♀ (*1*). Second, when the mutation rate is recorded separately for the four successive broods from treated ♂♂, the following figures are obtained: first brood, three lethals in 860 chromosomes; second and third broods, no lethals in 1543 chromosomes; fourth brood, one lethal in 203 chromosomes. This curve of mutation rate with a peak for the first sperm used and an increase for spermatozoa which have been stored for a considerable time, has been described by Lamy (*3*), and Muller (*4*).

As the feeding method only treats larvae, the negative result obtained on adults did not seem conclusive. Additional tests on larvae were carried out with a new apparatus which allowed milder and therefore longer exposures to be made. In the first experiments old larvae were used, because these can stand a fairly long exposure. However, when feeding tests, carried out at the same time, indicated a sensitive period to the mutagen early in the third instar, tests were also carried out on younger larvae of this age. These larvae can stand only a much shorter exposure without being killed or sterilized.

TABLE 2

THE EFFECT OF FORMALDEHYDE VAPOR ON MUTATION RATE IN THE GERM CELLS OF LARVAE

Time in hr after laying of eggs	Length of exposure	No. of tested X-chromosomes	Lethals	
			No.	%
96–100	2 hr	2126	2	0.1
about 70	2 hr	138	0	0
46–56	2 hr	169	1	
	70 min	408	1	
	50 min	436	1	
		1013	3	0.3

In all tests, a considerable proportion of the larvae died either during exposure or later on. The two sexes had the same survival rate. Table 2 summarizes the data gained from exposure of larvae.

In all series, the results are negative. Thus, all attempts to induce mutations by formaldehyde vapor have failed, and it seems most likely that the effective mutagen is a compound formed by reaction of formaldehyde with one of the components of the food. Experiments to identify this component have been started.

References

1. AUERBACH, C. *J. Genet.*, 1941, **41**, 255.
2. KAPLAN, W. D. *Science*, 1948, **108**, 43.
3. LAMY, R. *J. Genet.*, 1947, **48**, 223.
4. MULLER, H. J. *Yearb. Amer. Phil. Soc.*, 1945, 150.
5. RAPOPORT, J. A. *C. R. Acad. Sci. U. R. S. S.*, 1946, **54**, 65.

Inhibition of Phosphatases by Beryllium and Antagonism of the Inhibition by Manganese

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Beryllium poisoning has become a subject of considerable practical importance as a result of the use of beryllium compounds in the fluorescent light industry and in several other industrial processes. The occurrence of a number of cases of beryllium poisoning as a result of the industrial use of this metal has emphasized the necessity of obtaining detailed information on the toxicity and mode of action of beryllium in mammals. While numerous studies have been carried out on the toxicology of beryllium, little is known of its mechanism of action and no therapeutic measures for either acute or chronic beryllium poisoning are available.

In undertaking studies on the mechanism of action of beryllium, we were interested in examining its effect on enzymatic reactions with special attention being directed toward enzymes requiring metallic activators. The occurrence of beryllium in the same atomic group with calcium and magnesium and the many similarities in the chemical behavior of these three metals suggested that the toxic effects of beryllium might involve interference with the biological functions of calcium and magnesium. The present communication describes the results of experiments which demonstrate that beryllium inhibits alkaline phosphatases activated by magnesium and calcium, and that this inhibition can be prevented and re-

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versed by manganese.

In order to ascertain the action of beryllium on a calcium-activated enzyme, the adenosine triphosphatase system was employed, and the effect of beryllium on the adenosine triphosphatase activity of mouse liver and lung tissue was measured by the method of DuBois and Potter

TABLE 1

THE EFFECT OF BERYLLIUM ON THE ATP-ASE ACTIVITY OF MOUSE LIVER AND LUNG TISSUE

Tissue	Molar concn. of beryllium	ATP-ase units	% Inhibition
Liver	0	13.4	..
	6.1×10^{-3}	1.0	92
	3.0×10^{-3}	3.0	78
	8.0×10^{-4}	9.3	21
Lung	0	17.3	..
	6.1×10^{-3}	1.7	90
	3.0×10^{-3}	4.4	74
	8.0×10^{-4}	10.8	37

(*2*). For all of our tests, aqueous solutions of beryllium chloride were employed. The inhibitory action of beryllium on adenosine triphosphatase activity is shown by the data in Table 1, from which it may be seen that the activity of lung and liver tissue was inhibited to about the same extent by similar concentrations of beryllium.

TABLE 2

THE INHIBITORY ACTION OF BERYLLIUM ON ALKALINE PHOSPHATASE OF RAT TISSUES

Tissue	Phosphatase activity mg P/g tissue/hr	Molar concn. of Be for 50% inhibition
Serum	0.33	1.8×10^{-6}
Duodenum	34.20	5.0×10^{-6}
Kidney	32.6	5.9×10^{-5}
Lung	1.78	4.5×10^{-4}
Brain	0.72	5.9×10^{-4}

In view of the inhibitory action of beryllium on adenosine triphosphatase activity, we were interested in testing the effect of beryllium on magnesium-activated phosphatases. The similarity in the chemical behavior of magnesium and beryllium was first observed in 1888 by Sestini (*5*) who found that beryllium could replace magnesium in the growth of plants. Recently Grier, Hood, and Hoagland (*3*) and Klemperer, Miller, and Hill (*4*) have demonstrated that beryllium exerts a strong inhibitory action on alkaline phosphatases. To compare the inhibitory action of beryllium on phosphatases activated by magnesium and calcium, we have examined the effect of beryllium on the alkaline phosphatase activity of several tissues. Alkaline phosphatase measurements were performed using essentially the method of Bodansky (*1*) with modifications in the quantities of reactants to make the method suitable for small quantities of tissue. The test system contained 1 ml of 0.025 M Veronal buffer (pH 8.9) containing 0.015 M sodium β-glycerophosphate, 1–100 mg of homogenized tissue or serum, and enough water to make a final volume of 1.7 ml. Solutions of the various metals employed replaced an equivalent amount of water in the test system. After incubation for 30 min at 38° C, the reaction was stopped by the addition of 0.3 ml of 50% trichloracetic acid and after centrifugation the inorganic phosphate liberated was measured. Table 2 gives the alkaline phosphatase activity of several tissues

TABLE 3

ANTAGONISTIC ACTION OF MANGANESE ON THE INHIBITION OF SERUM PHOSPHATASE BY BERYLLIUM (5.9×10^{-10} M)

Molar concn. of manganese	% Inhibition
0	77
5.9×10^{-3}	4
5.9×10^{-4}	13
2.9×10^{-4}	51
5.8×10^{-5}	64

in the absence of added metallic ions. The values are expressed as mg of phosphorus liberated per g of tissue per hr together with the molar concentration of beryllium necessary to produce 50% inhibition of the alkaline phosphatase activity of each tissue. These results demonstrated the pronounced inhibitory action of beryllium on magnesium-activated phosphatases. There was a considerable difference in the sensitivity of the alkaline phosphatase of serum and various other tissues to beryllium, with serum phosphatases being the most sensitive of those studied.

It is likely that the inhibitory action of beryllium on phosphatases results from competition with magnesium and calcium for the same group on the enzyme with which they react. In this connection we were interested in ascertaining whether increasing the concentration of magnesium or addition of some other metallic ion to the system would decrease the amount of inhibition of serum phosphatase by beryllium. Various concentrations of magnesium as high as 5.9×10^{-3} M did not influence the inhibitory action of beryllium. This observation was in agreement with the results of other investigators (*3*). This indicated that beryllium has a much greater affinity for the enzyme than does magnesium. Since manganese also activates alkaline phosphatases, we tested the inhibitory action of beryllium on serum phosphatase in the presence of various concentrations of manganese as manganese sulfate. When manganese and beryllium were present in the test system at the time of addition of the serum, manganese markedly decreased the inhibition produced by beryllium, as illustrated by the data in Table 3. It was also found that manganese was effective in antagonizing the action of beryllium when added to the reaction mixture 5 min after the beryllium and serum were mixed. Cobalt and nickel also antagonized the inhibitory effect of beryllium on serum phosphatase *in vitro*.

The results of these experiments give support to the hypothesis that beryllium interferes in biological reactions in which magnesium and calcium participate. Magnesium-activated reactions appear to be more sensitive toward the action of beryllium than calcium-acti-

vated reactions. The extent to which the effects of beryllium on phosphatases are involved in acute and chronic beryllium poisoning must be determined by further experimentation on beryllium-poisoned animals. The ability of manganese to counteract the inhibitory effects of beryllium on phosphatases *in vitro* is encouraging because it provides evidence of the possibility of reversing the combination of beryllium with tissue constituents and thereby suggests a possible approach to the development of therapy for beryllium poisoning. In this connection, experiments are in progress at the present time in which the effects of several metals on acute and chronic beryllium poisoning are being tested. The results of these experiments will be reported in detail elsewhere.

References

1. Bodansky, A. *J. biol. Chem.*, 1937, **120**, 167.
2. DuBois, K. P. and Potter, V. R. *J. biol. Chem.*, 1943, **150**, 185.
3. Grier, R. S., Hood, M. B., and Hoagland, M. B. *J. biol. Chem.*, 1949, **180**, 289.
4. Klemperer, F. W., Miller, J. M., and Hill, C. J. *J. biol. Chem.*, 1949, **180**, 281.
5. Sestini, F. *Chem. Centr.*, 1888, **59**, 1622.

The Skin Temperature of an Extremity as a Measure of Its Blood Flow

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Skin temperature often is used as an index of blood flow in the extremities, even though the relation may be merely implicit. The purpose of this communication is to emphasize the fact, mentioned by Lewis (*3*) and others, that skin temperature is valid as a measure of extremity blood flow under certain conditions only.

Skin temperature depends upon both the rate of heat supply to the skin and the rate of heat removal—that is, upon the temperature and flow rate of the blood, and the insulation and temperature difference between skin and environment. If the temperature difference is small, heat will be removed so slowly that even large changes of blood flow will have little effect on the skin temperature. This is illustrated in the figures, in which are plotted finger blood flow and finger temperature against time. (Finger blood flow was measured with the plethysmographic method of Goetz (*2*) using air transmission. The values given are the averages of the flow at systole and at the end of diastole. Finger temperatures were measured with small thermocouples attached with adhesive tape, and connected to an instrument sensitive to 0.15° C and recording at 80-sec intervals. The hand was bare.) At a room temperature of approximately 32° C it is obvious from Fig. 1 that the finger surface temperature bears no relation to changes of blood flow. The correspondence is scarcely improved at an ambient temperature of about 21° C (Fig. 2). The air movement in both these experiments was the same.

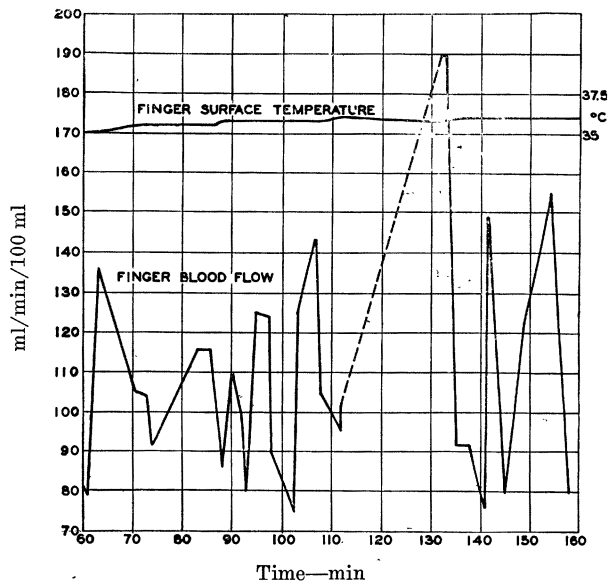

Fig. 1. Blood flow and skin temperature at 32° C ambient. Hand air insulation = 0.54 equivalent clo.

At about 7° C room temperature, with greater air movement, skin temperature follows the blood flow pattern fairly well, as can be seen in the example given in Fig. 3. At −34° C, with a gradient of 55–66° C between the bare hand and the ambient air, the lag between blood flow change and skin temperature change is probably negligible; for example, a very minor emotional disturbance may cause a fall of finger temperature of 8° C within 2 min; or a few deep breaths, of 3° C.

Our experience, exemplified by the figures, is that the heat loss rate calculated for the hand should be 12 kg-cal/hr (240 kg-cal/hr/m²) or more, if the skin tempera-

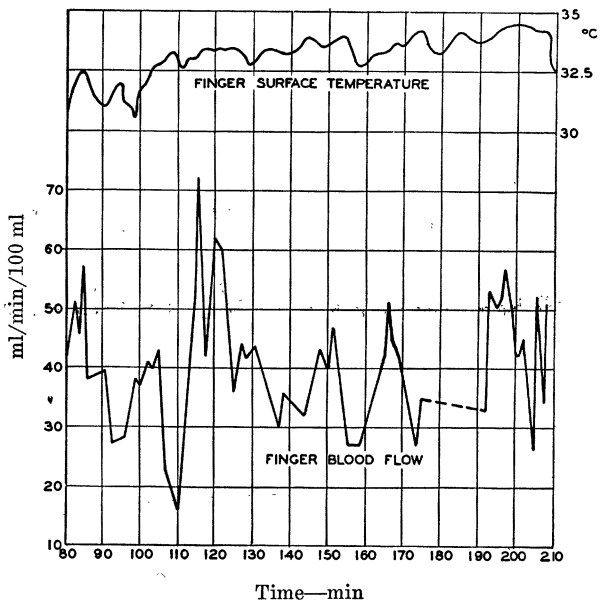

Fig. 2. Blood flow and skin temperature at 21° C ambient. Hand air insulation = 0.54 equivalent clo.