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Effect of Nicotinic Acid on Myocardial Systole, Coronary Flow, and Arrhythmias of Isolated Heart.

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The known role of nicotinic acid in carbohydrate metabolism, on which, of course, contraction of muscle largely depends, suggests that this material might have a favorable effect on various disturbances of myocardial function. Briefly to review the chemical processes accompanying contraction, it should be recalled that Lundsgaard[1] showed that the initial energy for the contractile mechanism is derived from the breakdown of phosphocreatine (Eggleton and Eggleton;[2] Fiske and Subbarow[3]) into phosphoric acid and creatine. In order for muscular activity to continue, phosphocreatine must be regenerated; and this resynthesis is accomplished by energy derived from the metabolism of carbohydrate. By a series of enzymatic changes (reviewed by Best and Taylor[4]), glucose is broken down into 2 triose compounds, one of which is 3-phosphoglyceraldehyde. It is with the oxidation of this triose that nicotinamide is concerned; this amide constitutes the prosthetic group of diphosphopyridine nucleotide, a coenzyme which, in the presence of a specific protein, accepts hydrogen from 3-phosphoglyceraldehyde and thus accomplishes its oxidation into phosphoglyceric acid. The latter then undergoes enzymatic rearrangements to form pyruvic acid (von Euler *et al.*[5])

From this point, the fate of the reduced pyridine nucleotide and of pyruvic acid varies depending on the presence or absence of oxygen. Under aerobic conditions the pyruvic acid is oxidized through the medium of thiamine and flavoprotein-cytochrome systems to carbon dioxide and water. At the same time, the reduced pyridine nucleotide is rapidly reoxidized and thus may participate over and over again in the primary oxidation of the original triose.

In the absence of oxygen, on the other hand, the direct reoxidation of the reduced pyridine nucleotide by oxygen is no longer possible. At the same time and for the same reason, pyruvic acid also cannot be removed by oxidation; and so, under these anaerobic conditions, the 2 interact, yielding lactic acid and the oxidized form of the pyridine nucleotide. Thus regenerated, the coenzyme is again available to act as a hydrogen acceptor and the anaerobic breakdown of carbohydrate can proceed until equilibrium conditions or acid formation call a halt to the process. (Reviewed by Ball[6]). Though this anaerobic mechanism represents incomplete combustion and succeeds in liberating only a part of the total energy available were oxygen present, its role in muscular contraction is crucial. Its effectiveness, moreover, obviously depends on an adequate supply of

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1 Lundsgaard, E., *Biochem. Z.*, 1930, **217**, 162; 1930, **227**, 51; *Ann. Rev. Biochem.*, 1938, **7**, 377.

2 Eggleton, P., and Eggleton, G. P., *Biochem. J.*, 1927, **21**, 190; *J. Physiol.*, 1927, **63**, 155; 1928, **65**, 15.

3 Fiske, C. H., and Subbarow, Y., *Science*, 1927, **65**, 401; *ibid.*, 1928, **67**, 169; *J. Biol. Chem.*, 1929, **8**, 629.

4 Best, C. H., and Taylor, N. B., *The Physiological Basis of Medical Practice*, Baltimore, The Williams and Wilkins Co., 2nd edition, 1939, 984.

5 von Euler, H., Adler, E., Günther, G., and Hellström, H., *Z. physiol. Chem.*, 1937, **245**, 217.

6 Ball, E. G., *Bull. Johns Hopkins Hosp.*, 1939, **65**, 253.

nicotinamide in the oxidized rather than the reduced form.

Glycolysis in cardiac muscle proceeds along lines similar to those of skeletal muscle. (Ochoa[7]). Nicotinamide is therefore as necessary for cardiac activity as it is for contraction of striated voluntary muscle; and that its deficiency might interfere with the function of the heart is to be expected. The observations of Feil,[8] Mainzer and Krause,[9] and Rachmilewitz and Braun[10] are in line with this theoretical implication. These authors detected electrocardiographic changes in from 50 to 75% of patients with pellagra, and in many instances were able rapidly to reverse the abnormalities by nicotinamide therapy. Were such changes confined simply to pellagrins, the usefulness of this form of treatment would be sharply circumscribed; but additional potentialities are opened by the discovery (Mann and Quastel[11]) that *damaged* tissues possess an agent which breaks down cozymase at a rapid rate. This may take place under strictly anaerobic conditions, and is due, according to the interpretation of Mann and Quastel, to hydrolysis of the coenzymes by a nucleotidase. The addition of nicotinamide to such inactivated systems results in a marked stimulation of oxygen uptake. It is known (Kohn;[12] Axelrod *et al.*[13]) that the administration of nicotinic acid is followed by an increase in the concentration of the pyridine nucleotides, and the possibility therefore arises that nicotinic acid or its amide might prove efficacious under a variety of conditions in which cardiac tissue has been damaged and the pyridine nucleotides consequently inactivated.

Direct investigations of the action of nicotinic acid and its amide on the heart have been few. Scaffidi and d'Agostino[14] concluded that the dilute acid has a vasodilator action on the coronary circulation but that stronger solutions cause vasoconstriction. They do not comment on the action of the drug on the myocardium itself, nor, indeed, is it clear from their description that the observed changes might not have been due to poor control of hydrogen-ion concentration.

These limited data on nicotinic acid itself are somewhat supplemented by the numerous studies that have been conducted on coramine, which is the diethylamide of nicotinic acid. In certain respects at least, coramine duplicates the physiological action of the latter (Smith[15]). Uhlmann[16] showed that coramine directly stimulates the perfused heart poisoned with chloral hydrate or choline. Using Starling's heart-lung preparations (which are essentially *normal* hearts), Leyko[17] found that coronary flow is increased, regardless of the state of contraction, which he found to be unaltered by small doses but depressed by stronger concentrations of coramine. By the same technic, Mezey[18] made the important distinction between its action on the normal heart as contrasted with that on the insufficient or poisoned myocardium. In the former, the effects of coramine are negligible, there being an increase in diastole with resultant increase in stroke volume; but a concomitant decrease in frequency causes the minute volume to remain the same. In contrast, in the *insufficient* heart there occur reduction of elevated venous pressure as measured in the right heart, increase in diastole with consequent lessening of venous stasis, rise in arterial pressure, and an in-

[7] Ochoa, S., *Biochem. Z.*, 1937, **290**, 62.

[8] Feil, H., *Am. Heart J.*, 1936, **11**, 173.

[9] Mainzer, F., and Krause, M., *Brit. Heart J.*, 1940, **2**, 85.

[10] Rachmilewitz, M., and Braun, K., *Am. Heart J.*, 1944, **27**, 203.

[11] Mann, P. J. G., and Quastel, J. H., *Nature*, 1941, **147**, 326; *Biochem. J.*, 1941, **35**, 502.

[12] Kohn, H. I., *Biochem. J.*, 1938, **32**, 2075.

[13] Axelrod, A. E., Madden, R. J., and Elvehjem, C. A., *J. Biol. Chem.*, 1939, **131**, 85; Axelrod, A. E., Gordon, E. S., and Elvehjem, C. A., *Am. J. Med. Sc.*, 1940, **199**, 697.

[14] Scaffidi, V., Jr., and d'Agostino, L., *Riv. pat. sper.*, 1939, **22**, 437.

[15] Smith, David T., Margolis, George, and Margolis, Lester H., *J. Pharmacol. and Exp. Therap.*, 1940, **68**, No. 4, 458.

[16] Uhlmann, F., *Z. ges. exp. Med.*, 1924, **43**, 556.

[17] Leyko, E., *J. Pharmacol. and Exp. Therap.*, 1930, **38**, 31.

[18] Mezey, K., *Klin. Woch.*, 1935, **14**, 1176; *Arch. exp. Path. u. Pharmakol.*, 1935, **177**, 235.

crease in minute volume. Coronary dilatation has been noted by Greene[19] and Stoland and Ginsberg;[20] and Cowan[21] has shown that increase in voltage of the electrocardiogram, elevations of the T-wave in leads I and II, and correction of preexisting hypotension may follow the administration of coramine.

Suggestive as these numerous observations are, it seemed worth while to investigate the action of nicotinic acid on the perfused heart, and to note especially its effect in those cases in which the myocardium was unable to function normally.

Experimental. Methods. Rabbits were killed by a quick blow on the head, and the heart was removed rapidly and mounted on a perfusion apparatus in such way that the perfusion fluid entered the aorta above the valves and was thus forced into the coronary vessels. The effluent was measured either in a graduate or in a small, subjacent pan so arranged that each 5 cc of fluid would cause the pan to trip, empty itself, and close an electrical circuit activating a signal on the kymograph. The temperature of the perfusion fluid was kept constant at 37°C by means of a water jacket regulated by a Bratton thermostat.[22] A modified Ringer-Locke solution was employed as a perfusion fluid, each liter containing the following: sodium chloride, 21.7 cc of a 30% solution; sodium bicarbonate, 23.0 cc of an 8% solution; potassium chloride, 4.7 cc of a 9% solution; anhydrous calcium chloride, 4.8 cc of a 5% solution. When a mixture of 5% carbon dioxide and 95% oxygen was bubbled through this solution, the pH remained constant at 7.4, and the buffering capacity was such that it would tolerate the addition of nicotinic acid in a concentration as high as 1:100,000 without change of hydrogen-ion concentration. Small hooks were inserted into the epicardium on opposite sides of the heart, one being fixed and the other connected by means of fine silk to a heart lever, the excursions of which were recorded kymographically.

TABLE I.
Effect of Nicotinic Acid on Heart Rate and Coronary Flow.

Type of perfusion fluid	Pulse	Perfusion rate in cc per min
Ringer's	52	2.7
Nicotinic Acid 1:1,000,000	76	10.8
Ringer's	74	8.2
Nicotinic Acid 1:500,000	84	8.3
Ringer's	72	5.7
Nicotinic Acid 1:100,000	72	5.0
Ringer's	36	2.5
Nicotinic Acid, 1:100,000	32	2.9

Forty experiments were performed according to the method outlined, and nicotinic acid was employed in concentrations ranging from 1:100,000 to 1:2,000,000. Nicotinic acid amide was used in 6 of the experiments.

Results. Effect on Heart Rate. The effect of nicotinic acid on the heart rate was not striking in any instance. In two-thirds of the 40 experiments, the rate was increased from an initial average of 64 to an average of 76. In 2 cases, there was no change. These alterations apparently were not significant, for they did not exceed the variations in rate observed from time to time when Ringer's solution alone was used as the perfusion fluid.

Effect on Rate of Coronary Flow. The rate of flow of the perfusion fluid through the coronary vessels was increased in 31 preparations, decreased in 3 and unaffected in 6 instances. A typical protocol of this effect is shown in Table I. The magnitude of increase was from 1½-fold to as much as 10-fold. In all except 3 cases, this augmentation of coronary flow was accompanied by an increase in the amplitude of the myocardial excursion or by a moderate increase in heart rate. The increased flow, therefore, cannot be interpreted as necessarily implying that dilatation of the coronary vessels had occurred; for the improved action of the heart could have been sufficient to account for the observed increase in coronary flow. Moreover, as indicated in Table I, the change in rate of flow in general was observed only once in a given preparation, subsequent applications of nicotinic acid to the same preparation being without effect.

Effect on Amplitude of Cardiac Excursion.

19 Greene, C. W., *J. Pharmacol. and Exp. Therap.*, 1936, **57**, 98.

20 Stoland, O. O., and Ginsberg, A. M., *J. Pharmacol. and Exp. Therap.*, 1937, **60**, 396.

21 Cowan, J. H., *Am. J. Med. Sc.*, 1937, **193**, 673.

22 Bratton, A. C., *Science*, 1939, **89**, 589.

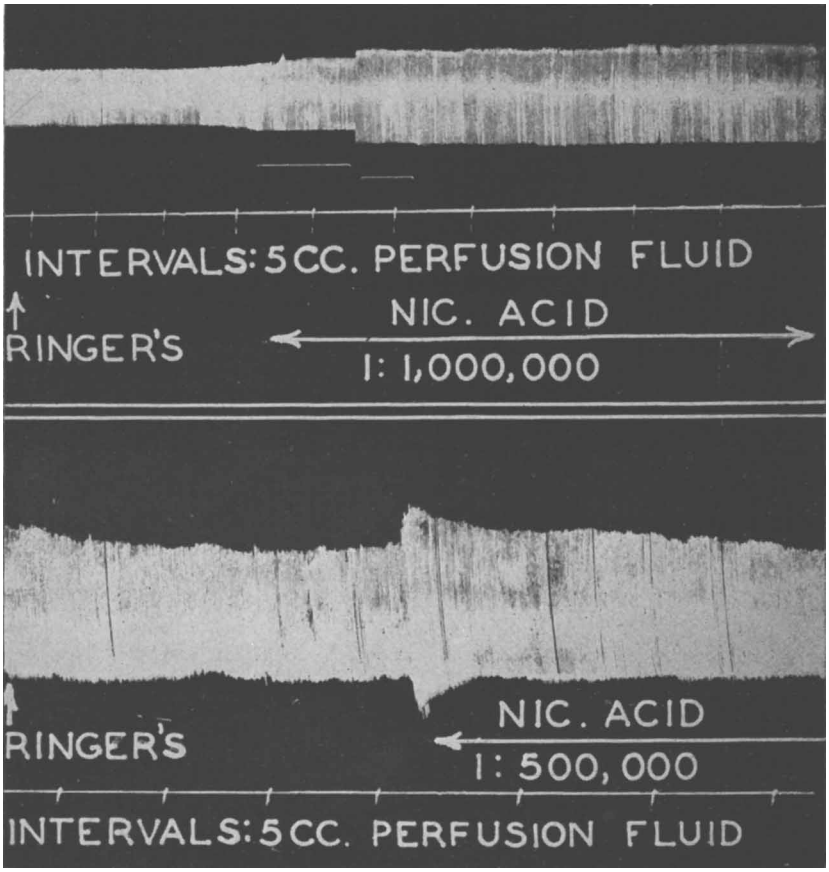

FIG. 1.
Effect of nicotinic acid on the amplitude of a poorly functioning heart.

In only 8 instances among the 40 experiments did nicotinic acid fail to augment the excursion of the cardiac muscle. This effect is shown graphically in Fig. 1, 2, and 3. As indicated in these kymographic tracings, this increase in amplitude was due principally to a more effective systole, there being only slightly greater diastolic relaxation than was observed with Ringer's solution alone.

In approximately three-fourths of the cases, this effect on systole was observed only once in a given preparation. When Ringer's solution was reapplied there was, of course, a slow and gradual decrease in cardiac excursion characteristic of preparations of this kind. Reapplication of nicotinic acid in such instances stimulated systole repeatedly in only about one-fourth of the cases. The most striking example of this recurring effect is illustrated in Fig. 2. This preparation varies from the usual in that 1 g glucose has been added to each liter of Ringer's solution.

Effect on Arrhythmia. One of the most impressive observations in the experiments was the abolition of certain arrhythmias by nicotinic acid. Spontaneous partial heart block was seen in 3 cases. The application of nicotinic acid in 2 of these immediately remedied the condition, while it was without effect in the third instance.

In 2 cases, ventricular standstill developed, apparently incident to technical delay in removing the heart from the chest cavity and mounting on the perfusion apparatus. In both instances, nicotinic acid was immediately effective in stimulating the myocardium to normal action (Fig. 4).

Four cases of spontaneous ventricular

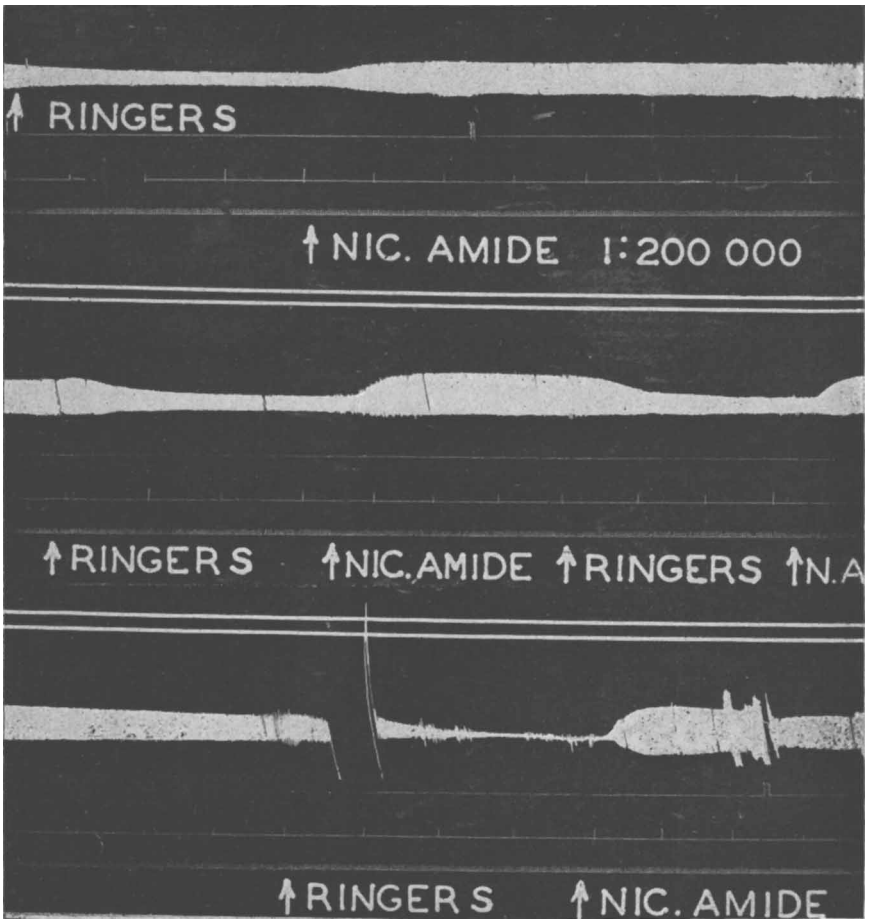

FIG. 2.
Repeated myocardial stimulation by nicotinamide. One percent glucose was incorporated in the perfusion fluid.

fibrillation were encountered in this series of experiments. Two of these were abolished by nicotinic acid (Fig. 5), while the other 2 were not affected. In one of these latter, both the fibrillation and its failure to respond favorably to nicotinic acid were probably explainable on the basis of thrombi which, it was discovered, had developed in major branches of the coronary arteries. Since the incidence of this abnormality as a spontaneous phenomenon is low, attempts were made in 2 preparations to reproduce the condition by electrical stimulation. In one of these experiments, electrical stimulation applied when the perfusion fluid contained 1:200,000 nicotinamide was capable of producing only very transient runs of fibrillation. Electrical stimulation in the absence of nicotinamide resulted in protracted fibrillation; reapplication of nicotinamide was followed promptly by the disappearance of this arrhythmia. In the other instance, nicotinamide in dilutions of 1:500,000 and 1:50,000 failed to abolish the abnormality.

Discussion. These beneficial effects of nicotinic acid on the failing myocardium demand an appraisal of the design and execution of our experiments in order to determine, if possible, the fundamental cause of the disturbances which proved remediable by nicotinic acid. Obviously, the abnormalities cannot be ascribed to a primary deficiency of this vitamin in the original preparation. One is forced, therefore, to search for factors

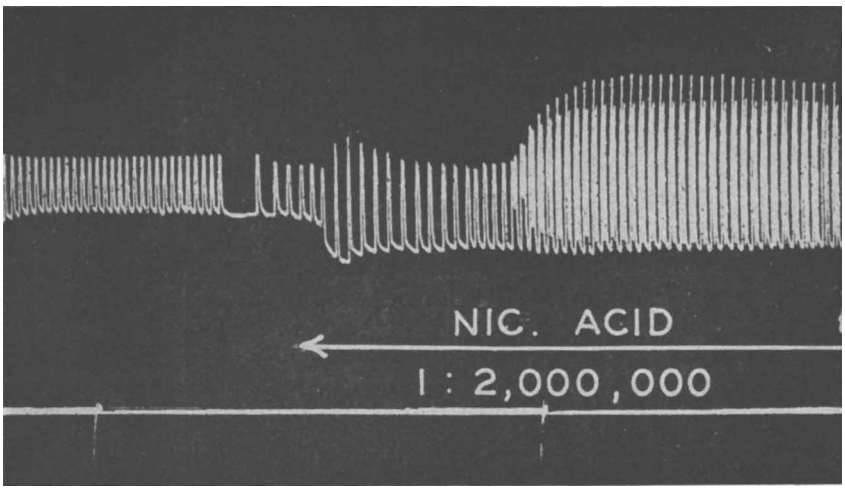

FIG. 3.
Effect of nicotinic acid on the dying heart. This preparation was 2 hours old.

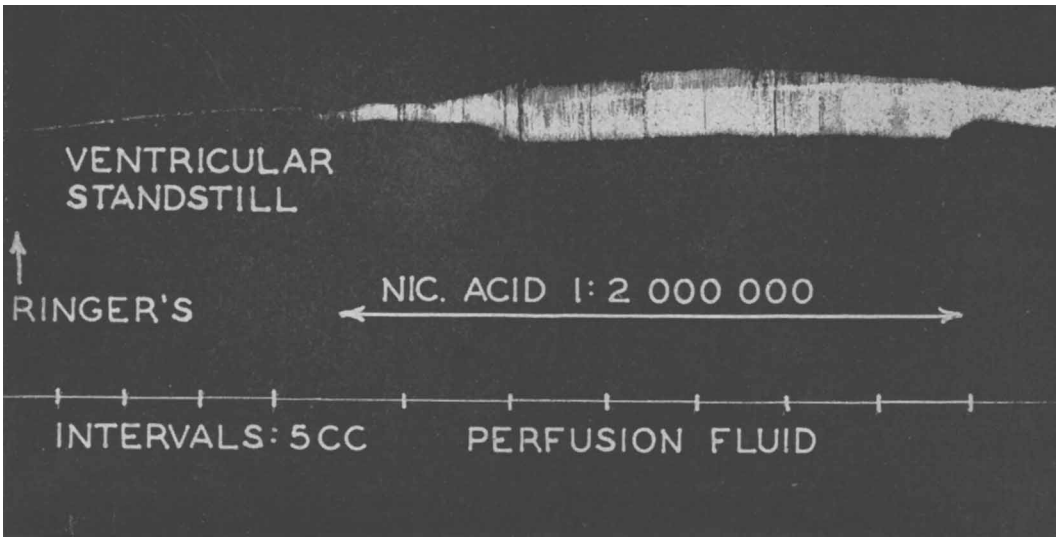

FIG. 4.
Effect of nicotinic acid on ventricular standstill.

that might lead to a temporary depletion or inactivation of diphosphopyridine nucleotide, of which nicotinamide is the prosthetic group.

On the basis of established biochemical principles 3 such factors come to mind: (1) Under the conditions of our experiments the heart was deriving its energy largely if not exclusively from carbohydrate. Sydenstricker[23] has stated that when carbohydrate is the principal source of energy, the pyridine nucleotides apparently are used up at a greatly increased rate. This circumstance, then, may have contributed to impairment of myocardial function. (2) The discovery of Mann and Quastel[11] noted above is of possible significance. It is conceivable, on this basis, that manipulative trauma induced the formation of a nucleotidase which could have accounted for hydrolysis of coenzyme with resultant cessation of glycolysis. (3) The

[23] Sydenstricker, V. P., *Arch. Int. Med.*, 1941, **67**. 746.

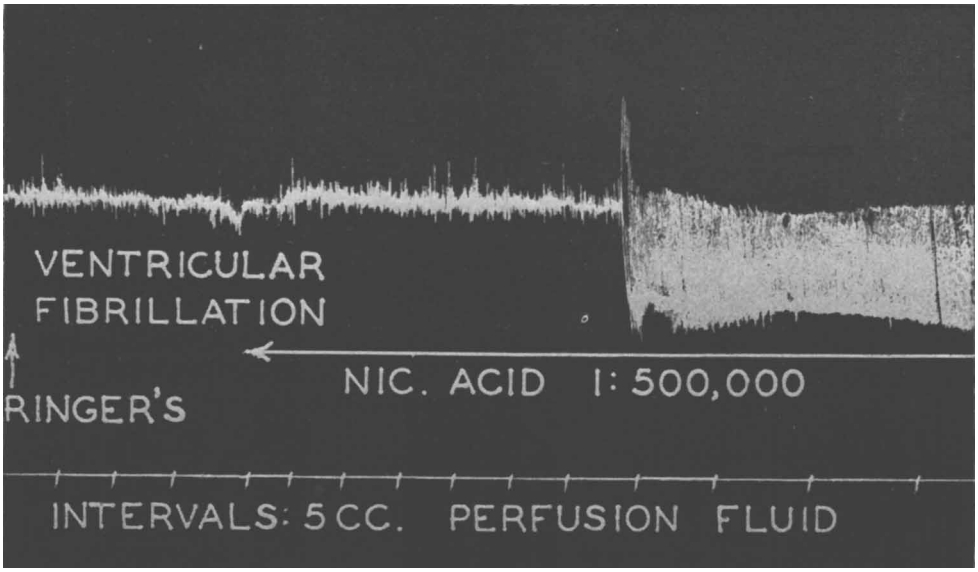

FIG. 5.
Effect of nicotinic acid on ventricular fibrillation.

most plausible explanation of the observed phenomena involves a consideration of the consequences of the temporary anoxia incident to technical delay in mounting the heart on the perfusion apparatus. It was held formerly (Meyerhof;[24] Warburg[25]) that the energy exchanges involved in enzymatic cellular respiration were entirely independent of oxygen tension and that they proceeded normally so long as the smallest amount of oxygen was present. Kempner,[26] however, was able to disprove this concept. Working with a variety of animal and bacterial cells, as well as with cell-free plasma, Kempner established definitely that if the oxygen tension decreases to very low levels, cellular respiration declines in rate and changes qualitatively, as evidenced by a marked fall in the respiratory quotient. The region of oxygen tension in which these changes are manifest, moreover, is above that at which anaerobic glycolysis begins. From what is known of the mechanisms by which cellular oxidation is mediated, this failure of cellular respiration seems to be due to the fact that the lack of oxygen impedes the rate at which the reduced catalysts are reoxidized. That cardiac function should suffer under the conditions of our experiments is therefore not surprising.

If, as seems entirely probable, the observed disturbances of cardiac function were actually due to inactivation or depletion of the pyridine nucleotides, it seems reasonable to believe that these temporary deficiencies are correctable by the addition of nicotinic acid or its amide to the perfusion fluid. Thus replenished, adequate coenzyme would be available for the resumption of anaerobic glycolysis, equilibrium between pyridine nucleotides and the other respiratory enzymes would be reestablished, and the aerobic phases of oxidation could then proceed in normal and orderly fashion.

Summary. Experiments designed to in-

[24] Gildemeister, M., and others, editors, Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere. Herausgegeben von M. Gildemeister, R. Goldschmidt, C. Neuberg, J. Parnas und W. Ruhland. Band XXII: Die chemischen Vorgänge in Muskel und ihr Zusammenhang mit Arbeitsleistung und Wärmebildung, von Otto Meyerhof. Berlin, 1930, Julius Springer.

[25] Warburg, O., and Kubowitz, F., *Biochem. Z.*, 1929, **214**, 4.

[26] Kempner, W., *Cellular and Comp. Physiol.*, 1937, **10**, 339; *Cold Spring Harbor Symposia on Quantitative Biology*, 1939, **7**, 269.

vestigate the action of nicotinic acid on the isolated rabbit's heart are reported. Dilutions of from 1:100,000 to 1:2,000,000 were employed, and careful attention was paid to constancy of temperature and hydrogen-ion concentration.

It was found that the effect of nicotinic acid on well functioning hearts is insignificant. In the case of failure of the myocardium, however, this pyridine derivative causes a marked increase in amplitude of the cardiac excursion, reversal of abnormal rhythms (including partial heart block, ventricular fibrillation, and ventricular standstill), and at times a considerable augmentation of coronary flow. In no case does it appear to have an unfavorable influence.

The explanation of these effects may lie in the role played by nicotinic acid in carbohydrate metabolism. The idea is advanced that the observed disturbances of myocardial action are due to inactivation or depletion of the pyridine nucleotides by the anoxia incident to experimental technic; and that restoration of normal function probably depends on correction of this temporary and reversible deficiency by the addition of nicotinic acid to the perfusion fluid.

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Inactivation of Influenza Virus with Sulfur and Nitrogen Mustards.

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It has been demonstrated that sulfur and nitrogen mustards react chemically with a variety of proteins and enzymes of biological importance, and in so doing alter their physical properties and physiological functions. Presumably it is by such a mechanism of action that mustards abolish the infectivity of the nucleoprotein plant viruses causing tobacco mosaic and bushy stunt disease.[1] Recently, Tenbroeck and Herriott[2] have shown that the animal viruses of eastern equine encephalomyelitis, fixed rabies and hog cholera also may be rendered noninfectious by treatment with sulfur mustard without, however, altering their antigenicity. These mustard-inactivated viruses provoked a specific, protective immune response when injected into animals, suggesting the practical application of this method for the preparation of killed viral vaccines.

The present report deals with an investigation of the effects of sulfur mustard, bis-betachloroethyl sulfide (HS), and nitrogen mustards, bis- and tris-betachloroethyl amine (HN), on influenza virus. It will be shown that both types of mustard destroy the ability of this virus to infect susceptible animals, under appropriate conditions, but that high concentrations of these substances fail to alter its hemagglutinative properties.

Experimental. Effect of HS on Infectivity of Influenza Virus. The influenza virus employed was the PR8 strain in mouse lung passage. Dilutions of the virus in nutrient broth were made from 10% suspensions of infected lung, and the LD_{50} was found to be approximately .05 cc intranasally of a 10^{-6} dilution.

An approximately saturated solution of HS was prepared by adding 0.1 cc of HS to 50 cc of ice-cold 0.85% saline in a 250 cc stoppered flask, shaking vigorously by hand for one minute, and then placing the mixture in the refrigerator for 5 minutes to allow the undissolved residue of HS to separate and settle on the bottom of the flask. The assumption was made that the concentration of HS in the supernatant saline approached

1 Gilman, A., and Philips, F. S., *Science*, 1946, **103**, 409.

2 Tenbroeck, C., and Herriott, R. M., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 271.