BODILY REACTIONS TO TRAUMA. A POSSIBLE ROLE OF NUCLEOTIDES IN CARDIAC ISCHAEMIA.

H. B. STONER, H. N. GREEN AND C. J. THRELFALL.

From the Department of Pathology, University of Sheffield.

Received for publication August 7, 1948.

IN previous work on muscle ischaemia we have been concerned with the local and general changes produced by ischaemia of voluntary muscle. We have shown that after a period of muscle ischaemia there is an increase in the level of some components of the nucleotide molecule in the peripheral blood (Stoner and Green, 1944, 1945*a*). Examination of the muscle during and after ischaemia showed that the content of the various nucleotide fractions was low, and that this decrease was greater than could be accounted for by anoxaemic decomposition (Green, Bielschowsky and Stoner, in preparation). These findings, considered together, indicate, at the least, that fragments of the nucleotide molecule are liberated from voluntary muscle after ischaemia. However, it is not possible from the data thus obtained to decide in what form, or forms, the nucleotide becomes extracellular under these conditions.

The adenine nucleotide of cardiac muscle is thought to be present as diadenosine pentaphosphate (Beattie, Milroy and Strain, 1934). This polynucleotide is also found in voluntary muscle (Bielschowsky, M., unpublished result). The nucleotide content of cardiac muscle is also somewhat less than that of voluntary muscle. Despite these differences there is no evidence to suggest that nucleotide metabolism in cardiac muscle is essentially dissimilar from that in voluntary muscle. Since coronary occlusion is such an important pathological event, it is natural that we should include in our studies an investigation of the effect of ischaemia on cardiac muscle. At the outset it appeared that the essential problem was whether ischaemia leads to the release of nucleotide from cardiac muscle, but as the work proceeded other objectives entered the picture. The problem of the changes in the nucleotide content of cardiac muscle during and after ischaemia has been studied by direct methods similar to those used previously and by the indirect method of comparing the electrocardiographic changes produced by coronary occlusion and by the topical application of nucleotides and other substances to the heart.

METHODS.

Experiments were carried out on a total of 116 guinea-pigs, 48 rabbits, 3 cats and 5 toads (*Xenopus laevis*).

Experiments designed to test directly the possible liberation of nucleotide derivatives from the heart by ischaemia were carried out on the isolated heart of the rabbit perfused at 37° C. in the usual way with oxygenated Krebs-Henseleit (1932) Ringer's solution containing 0·1 per cent glucose. The perfusion pressure

was 60 cm. of perfusion fluid. The rate of coronary flow was determined with a modified Gaddum outflow recorder. In some cases, however, this outflow was used to perfuse a guinea-pig's heart by the technique of Bennet and Drury (1931), and the electrogram of this guinea-pig's heart was recorded with a Sanborn Viso-Cardiette type of electrocardiograph. Cardiac anoxaemia was produced by occlusion of branches of the left coronary artery by a ligature or clip. The standard period of anoxaemia was 1 hour when the experiment was terminated, or the circulation through the unperfused part of the heart restored by removing the clip and perfusing for a further 30 minutes. In some experiments coronary occlusion was continued for $1\frac{1}{2}$ hours in order to control the changes found in the muscle examined 30 minutes after 1 hour occlusion. Normal control muscle was obtained from hearts which had been perfused for 1 hour without any interference with the coronary flow. Samples of the outflow from the rabbit's heart were collected before, during and after coronary occlusion. Any enzymes which might be present in the sample were inactivated by boiling, and the heat-precipitated protein removed by filtration through Whatman No. 42 filter-paper. These solutions were then analysed. Before taking the specimens of ventricular muscle the anoxaemic area was outlined by the injection of 0·5 ml. of 2 per cent aqueous solution of Berlin Blue into the perfusion cannula whilst the coronary occlusion was still present. At the termination of the experiment the unstained area of the myocardium was rapidly excised, blotted and plunged into liquid air. The specimen was weighed and extracted with 10 per cent trichloroacetic acid (10 ml. per g. wet weight), as described by Davies, Francis and Stoner (1947), and the extracts analysed.

In some experiments the effect of autolysis on the nucleotide content of cardiac muscle was determined. For these experiments the heart was rapidly excised and a portion of the left ventricle blotted, immersed in liquid air and then extracted as above. The remainder of the heart was moistened with Krebs-Henseleit Ringer's solution and incubated at 37° C. for 1 hour, when the rest of the left ventricle was treated in the same way.

The analytical methods used were as follows: Phosphate (total, inorganic and 7 minutes) by Brigg's method (Peters and Van Slyke, 1932), pentose (total and phosphorylated) by a modification of Mejbaum's (1939) method (Green, Bielschowsky and Stoner, 1947), and the adenosine equivalent by the guinea-pig atrium method of Drury, Lutwak-Mann and Solandt (1938) as modified by us (Stoner and Green, 1944). Some of the extracts were also examined with the Beckman spectro-photometer in the U.V. range 230–300 mμ.

The indirect experiments consisted in a comparison of the changes in the form of the electrocardiogram (ECG) produced by cardiac ischaemia, and by the topical application of certain organic and inorganic compounds to the heart. Guinea-pigs were mainly used, but on occasion so were rabbits, cats and toads. All the mammals were anaesthetized with nembutal. The trachea was cannulated and the animal artificially respired. The anterior part of the chest wall with the lower part of the sternum was removed after tying ligatures round the ribs and the internal mammary arteries. The pericardium was incised and the heart laid bare. In the cat the arterial blood pressure was recorded in the carotid artery with a mercury manometer. The toads were pithed and the heart exposed in a similar manner. The ECG was recorded with a Sanborn Viso-Cardiette type of electrocardiograph through the three normal leads. The effect of the test

substances on the form of the ECG when injected beneath the visceral layer of the pericardium through a fine needle was studied, and compared with the changes produced by ligature of branches of the coronary arteries. The maximum volume of fluid injected in this way was 0·10 ml. in the guinea-pig, 0·15 ml. in the cat, and 0·10 ml. in the rabbit. The usual volume of fluid injected in the guinea-pig was 0·02–0·03 ml. In some instances the test substances were applied to the surface of the ventricles by the filter-paper method of Kisch, Nahum and Hoff (1940). The size of the filter-paper was roughly 5 mm. square.

The following nucleotides were used: the magnesium and sodium salts of adenosine triphosphate (ATP), the sodium salts of adenosine diphosphate (ADP), muscle adenylic acid (A5MP), inosine triphosphate (ITP) and cytidylic acid. Adenosine (British Drug Houses, Ltd.) was also used. The cytidylic acid was prepared from yeast nucleic acid, and the other compounds were prepared from BaATP (Boots). The purity of these compounds was not less than 98 per cent. Other organic compounds used were histamine acid phosphate, acetylcholine and sodium lactate. The inorganic compounds used were NaCl, $Na_4P_2O_7 . 10H_2O$, $Na_2HPO_4 . 12H_2O$, KCl, $MgSO_4$ and $CaCl_2$.

It proved necessary to determine the effect of some of these compounds on the coronary circulation in the guinea-pig, and also to determine the nucleotide content in the guinea-pig heart. Similar techniques to those described above for the rabbit were used.

RESULTS.

I. *Perfusion experiments.*

The perfusion experiments were designed to test the possibiilty that nucleotide or its breakdown products are liberated from heart muscle damaged by ischaemia. The nucleotide contents during and after a period of anoxaemia were compared with those in the normal perfused heart, and the perfusates were examined for the presence of nucleotide derivatives. Perfusion of the heart by itself decreased its nucleotide content. We previously found that the left ventricular muscle of the rabbit contains 2·05 $\pm$ 0·4 mg. adenosine per g. wet weight and 0·19 $\pm$ 0·05 mg. "7 minute" P per g. wet weight immediately after its removal from the body (Davies *et al.*, 1947). The initial levels obtained in the present experiments are shown in Table III. The present observations show that the nucleotide content of the myocardium immediately after its removal from the body is distinctly higher than at the end of an hour's perfusion (Tables I and III). This difference may be explained in part by contamination of the unperfused samples with blood elements. This may not wholly account for the difference, since previous results (Davies *et al.*, 1947) obtained on hearts rapidly perfused free of blood were still somewhat higher, especially in regard to their "7 minute" P content, than those now obtained after perfusion for 1 hour. It was not to be expected that the perfused heart would be a completely normal structure, and considering the rather drastic nature of the experiments the results obtained for the different fractions are reasonably constant. The phosphate fractions are particularly constant. A certain amount of nucleotide must, however, be destroyed in the setting up and perfusion of the heart, and these findings indicated the necessity of comparing results in hearts which had been perfused for the same length of time.

TABLE I.—*The Adenosine, Pentose and Phosphate Contents of the Normal Left Ventricular Muscle of the Rabbit's Heart After Perfusion for* 1 *hour. Values expressed in mg. per g. wet weight.*

Rabbit number.	Adenosine equivalent.	Pentose.		Percentage phosphorylated.	Phosphate.		
		Total	Phosphorylated.		Total.	Inorg.	7 min.
50	1·8	1·15	1·13	97	0·76	0·44	0·11
51	1·8	1·31	1·04	79	0·77	0·37	0·10
54	0·6	1·08	0·78	72	0·74	0·35	0·13
56	1·0	2·14	2·10	98	0·85	0·39	0·10
62	0·8	0·86	0·69	80	0·65	0·29	0·10
81	0·6	0·77	0·61	79	0·76	0·39	0·14
Average	1·1	1·22	1·06	84	0·77	0·37	0·11

Occlusion of the left coronary artery produced further changes in the nucleotide and phosphate fractions of the myocardium of the left ventricle. The values thus obtained at the end of a 1-hour period of occlusion are given in Table II, and should be compared with those in Table I since the total perfusion time is the same in each case. This period of anoxaemia, whilst producing no outstanding changes in the adenosine and total pentose contents, consistently decreased the proportion of pentose in the phosphorylated form. Similarly in the case of the phosphate fractions, whilst the amount of total P was unchanged, there was a decrease in the " 7 minute " P content with a corresponding increase in the inorganic P content. These results represent a breakdown of fully phosphorylated nucleotide such as ATP to the lower members of the series, without any loss of the integral components of the molecule.

TABLE II.—The *Adenosine, Pentose and Phosphate Contents of the Left Ventricular Muscle of the Rabbit's Heart Perfused for* 1 *hour After Occlusion of the Left Coronary Artery. Values expressed in mg. per g. wet weight.*

Rabbit number.	Adenosine equivalent.	Pentose.		Percentage pentose phosphorylated.	Phosphate.		
		Total.	Phosphorylated.		Total.	Inorg.	7 min.
48	1·4	1·39	0·89	64	0·95	0·59	0·06
52	1·6	0·92	0·74	80	0·79	0·51	0·03
53	1·8	1·17	0·84	72	0·77	0·44	0·11
58	1·0	2·45	1·36	56	—	—	—
60	0·6	1·05	0·63	60	0·76	0·45	0·13
82	0·4	0·64	0·50	78	0·64	0·41	0·07
84	0·8	0·92	0·72	78	0·80	0·47	0·08
86	0·6	0·77	0·44	56	0·83	0·47	0·08
91	1·0	0·99	0·78	79	0·82	0·44	0·07
92	0·8	1·09	0·79	72	0·68	0·34	0·11
93	0·4	0·79	0·43	54	0·65	0·30	0·07
94	0·6	1·12	0·82	73	0·79	0·35	0·15
98	0·7	1·01	0·64	63	0·77	0·41	0·09
Average	0·9	1·10	0·74	68	0·77	0·43	0·09

TABLE III.—*The Adenosine, Pentose and Phosphate Contents of Rabbit Left Ventricular Muscle Before and After Autolysis at 37° C. for 1 hour. Values expressed in mg. per g. wet weight.*

Rabbit Number.	Adenosine Equivalent.		Pentose.				Phosphate.				"7 min."	
			Total.		Phosphorylated.		Total.		Inorg.			
	Before autolysis.	After autolysis.	Before autolysis.	After autolysis.	Before autolysis.	After autolysis.	Before autolysis.	After autolysis.	Before autolysis.	After autolysis.	Before autolysis.	After autolysis.
55	1·5	0·4	2·09	1·40	1·40	0·70	1·24	1·05	0·59	0·59	0·23	0·09
57	0·9	0·4	—	—	—	—	—	—	—	—	—	—
59	1·3	0·4	2·72	2·89	1·91	2·16	0·98	1·07	0·42	0·79	—	0·01
61	1·4	0·6	1·98	1·53	1·35	0·79	1·06	1·10	0·48	0·62	0·21	0·11
83	—	0·4	1·69	1·27	1·33	0·84	0·58	1·03	0·43	0·72	0·20	0·02
85	—	0·6	1·61	1·14	1·23	0·66	1·25	1·23	0·53	0·83	0·22	0·06
Average	1·3	0·5	2·02	1·64	1·44	1·03	1·02	1·09	0·49	0·71	0·22	0·06
Percentage change after autolysis	—	−62	—	−19	—	−28	—	+7	—	+45	—	−73

It is interesting to compare these changes with those which occurred during a similar period of autolysis (Table III). Under these conditions the breakdown of nucleotide was much more severe, and there was a striking fall in the " 7 minute " P level with an associated increase in the inorganic P content. The most striking change in the pentose content was in the phosphorylated fraction, which in all but one experiment was greatly reduced. This is in line with the phosphate changes. In addition there was a marked decrease in the adenosine content, and in this respect these results differ from those obtained after coronary occlusion (Table II). Otherwise the trend of events was similar under the two conditions.

If perfusion was continued for a further 30 minutes with the artery occluded no further alteration in the level of the various fractions occured, and the picture at the end of 90 minutes' anoxaemia was the same as after occlusion for 60 minutes. However, if the circulation through the left ventricle was restored at the end of 1 hour and perfusion continued for a further 30 minutes changes did occur. An obvious improvement in the heart beat occurred immediately after the removal of the occlusion, and this was to some extent reflected in the biochemical findings (Table IV). The total P content of the myocardium remained unaltered, but the " 7 minute " P level increased with a slight fall in the inorganic P content. Corresponding to the rise in " 7 minute " P level a greater proportion of the pentose was in the phosphorylated form, but lower values were obtained for the total pentose and adenosine contents. These results possibly indicate that some resynthesis of nucleotide occurs on restoration of the circulation.

TABLE IV.—*The Adenosine, Pentose and Phosphate Contents of the Left Ventricular Muscle of the Rabbit Sampled after Perfusion for* 90 *minutes. During the first* 60 *minutes of the perfusion the left coronary artery was occluded. Values expressed in mg. per g. wet weight.*

Rabbit number.	Adenosine equivalent.	Pentose.		Percentage phosphorylated.	Phosphate.		
		Total.	Phosphorylated.		Total.	Inorg.	7 min.
87	0·6	0·66	0·62	90	0·83	0·48	0·12
88	0·4	0·72	0·57	80	0·83	0·40	0·10
89	0·3	0·59	0·44	74	0·72	0·42	0·07
97	0·4	0·75	0·53	70	0·70	0·33	0·14
96	0·8	0·86	0·75	87	0·78	0·41	0·12
95	0·4	0·84	0·56	67	0·73	0·36	0·09
Average	0·5	0·73	0·50	78	0·77	0·40	0·12

The constancy of the total P content of the myocardium both during and after the period of anoxaemia gives no support for the idea that nucleotide components are liberated from the myocardium under these circumstances. This was supported by chemical and spectrophotometric observations on the perfusate during and after the period of occlusion. Normally the perfusate does not contain any nucleotide or its derivatives capable of detection by these methods, nor did any appear as the result of ischaemia. The only change detected in the perfusate under these conditions was an increase in the heat-precipitated protein. Any rapid liberation of nucleotide would probably be revealed by these methods,

TABLE V.—*The Effect of the Topical Application of Various Substances and of Acute Coronary Occlusion on the Level of the ST Segment in the Guinea-pig under Nembutal Anaesthesia together with the Influence of the Intravenous Injection of $MgSO_4$, Atropine, Prostigmin, Pyranisamine Maleate and Theophylline Ethylene Diamine (for doses see text) on the Changes so Produced. The dose given in the second column is the minimal effective dose in the case of compounds which displaced the ST segment and the maximum dose used in the case of other compounds. † = Compound dissolved in 0·85 per cent NaCl ; * = dose in terms of free acid.*

Compound.	Dose.	Alteration in ST segment.	Effect of compound after intravenous injection of—				
			$MgSO_4$.	Atropine sulphate.	Prostigmine.	Pyranisamine maleate.	Theophylline ethylene diamine.
† NaATP } (20 mg./ml.)	0·2 mg.	+	Reduced	Unaltered	—	—	Unaltered
† MgATP }							
† NaADP (20 mg./ml.)	0·6 mg.	+	Reduced	—	—	—	—
† NaA5MP (20 mg.*/ml.)	0·8 mg.*	+ slight	—	—	—	—	—
† Adenosine (10 mg./ml.)	0·5 mg.	nil	—	—	—	—	—
† NaITP (20 mg./ml.)	0·9 mg.	nil	—	—	—	—	—
† Na cytidylate (20 mg.*/ml.)	0·8 mg.*	nil	—	—	—	—	—
† Histamine acid phosphate (2 mg./ml.)	20 μg.	+	—	—	—	Reduced	—
† Acetylcholine hydrochloride (1 mg./ml.)	10 μg.	+ variable.	—	Reduced	Increased	—	—
Na lactate (0·154 M)	0·8 mg.	nil	—	—	—	—	—
Na_2HPO_4 (neutral 0·1 M)	0·1 ml.	+	Unaltered	—	—	—	—
$Na_4P_2O_7$ (neutral 0·1 M)	0·03 ml.	+	Slight reduction	—	—	—	—
KCl (0·15 M)	0·02 ml.	+	Unaltered	Unaltered	—	—	—
$CaCl_2$ (0·10 M)	0·04 ml.	+	Unaltered	—	—	—	—
$MgSO_4$ (0·154 M)	0·05 ml.	nil	—	—	—	—	—
NaCl (0·154 M)	0·10 ml.	nil	—	—	—	—	—
Coronary artery ligation	—	+	Reduced	Unaltered	Unaltered	Unaltered	Unaltered‡

‡ Unless initial change slight when T wave reversed.

since, where 10 mg. NaATP was injected into the anoxaemic myocardium, nucleotide was readily detected in the perfusate during the following 15 minutes.

The absence of any alteration in the perfusate was confirmed biologically both when the perfusate was tested on the guinea-pig atrium preparation and when it was passed directly through a second heart (guinea-pig). The guinea-pig atrium preparation will detect as little as 1 μg. adenosine, and control double perfusion experiments showed that the addition of 25 μg. to the funnel below the rabbit's heart gave a definite prolongation of the PR interval in the guinea-pig's heart, so that these methods are sufficiently sensitive to show the presence of significant amounts of adenyl compounds.

II. *Electrocardiographic experiments.*

Cardiac ischaemia in both man and animals is associated with well-recognized changes in the ECG, one of the more important of which is displacement of the ST segment. The exact interpretation of these changes is not known, but it is agreed that deviation of this segment is associated with the presence of ischaemic cardiac muscle rather than with the presence of necrotic or dead muscle or its fibrous tissue replacement (Katz, 1946). ST displacement is thought to arise from depolarization of part of the surface of the heart with the setting up of a current of injury (Katz, 1947), but no biochemical explanation has been offered for these electrical changes.

In previous work on the effect of ATP on the ECG, displacement of the ST segment was occasionally seen after its intravenous injection in man and animals (Wayne, Goodwin and Stoner, 1948). This was rather surprising, as ATP is a coronary vasodilator. We therefore postulated that this displacement might be due to a direct action of ATP and other nucleotides on the myocardium. The effect of applying these substances directly to the heart muscle was accordingly studied. Under these conditions much more striking changes occurred in the ST segment. The majority of these experiments were performed on the guinea-pig, and the results refer to that animal unless otherwise stated. Table V gives a summary of the main results.

Topical application of adenyl compounds.

The Na and Mg salts of ATP were injected into the superficial layers of the myocardium just beneath the visceral layer of the pericardium in the area of the left ventricle shown in Fig. 1 in doses varying from 0·005–0·8 mg. The minimal effective dose was 0·2 mg., and the standard dose was 0·4 mg. (0·02 ml. solution).

Within a few seconds of the injection the ventricular contractions became infrequent and abnormal, with long periods of ventricular asystole which led to dilatation of the right side of the heart. The period of major disturbance of the ventricular rhythm lasted from 6 to 21 seconds, according to the dose of ATP, at the end of which the contractions became more frequent and regular rhythm was restored. With the return of regular rhythm the engorgement of the right side disappeared. ECG records showed that during the periods of ventricular asystole atrial contractions continued, although at a somewhat slower rate. The first irregular ventricular contractions were due to ventricular extrasystoles, often giving bizarre QRS complexes. Normal rhythm returned through a short

period of heart block with delayed PR conduction. Intraventricular block was seen in the early irregular beats and during the early stages of the period of heart block, but not after the return of regular rhythm. Although regular rhythm was soon restored the ECG did not at once return to its normal form, and the changes in its shape, to be described in detail in the next paragraph, persisted

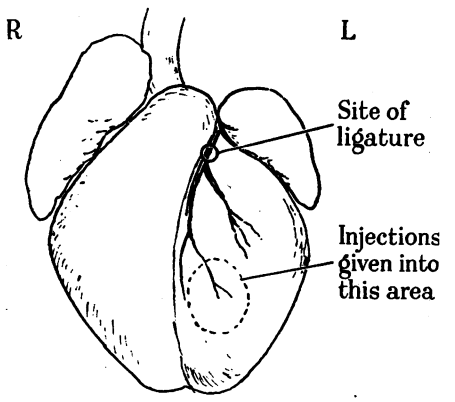

FIG. 1.—A diagram of the guinea-pig's heart to show the usual sites of injection and of coronary occlusion.

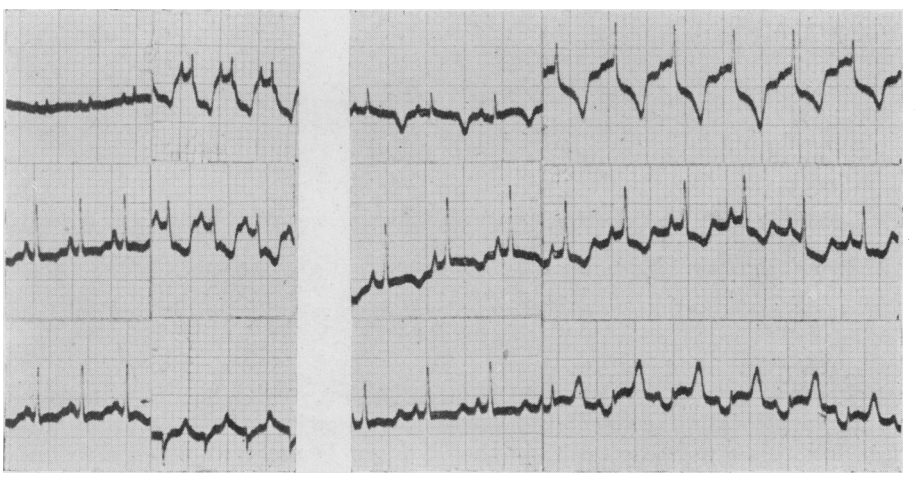

FIG. 2.—Effect of the injection of adenosine triphosphate into the surface of the left ventricle on the ECG of the guinea-pig under nembutal anaesthesia.
Guinea-pig 25/48 A = before ;
B = after the topical injection of 0·4 mg. MgATP in 0·02 ml.
Guinea-pig 17/48 C = before ;
D = after the topical injection of 0·4 mg. MgATP in 0·02 ml.

for 3–5 minutes, after which the ECG record appeared normal both in rhythm and form. Several injections could usually be given in the same animal before irrecoverable changes were produced, although in 3 of the 37 guinea-pigs receiving ATP death, with congestive cardiac failure, occurred after the first injection.

The most striking change in the shape of the ECG after the topical application of ATP was displacement of the ST segment (Fig. 2). This effect was very constant, and was seen in all but one of 37 guinea-pigs. It did not occur immediately after the injection, but became most evident when normal ventricular rhythm returned, and persisted for 3–5 minutes, when to outward appearances normal ventricular contractions were occurring. The degree of displacement of the ST segment was variable, but in the majority of cases was gross. The only condition for its appearance was that the ATP solution should be in direct contact with the surface of the heart. Injections given deeply into the myocardium had little effect on the form of the ECG. Although the injections were always given into approximately the same area of the heart, the direction of the ST displacement varied. In some animals it was elevated in Lead I, depressed in Lead III, with little change in Lead II; in others it was displaced in the same direction in all three leads, whilst in others only one lead was affected. Nor did the ST deviation always remain in the same direction after a single application of ATP.

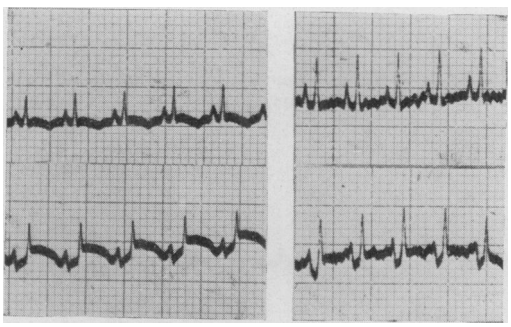

FIG. 3.—Shows the accentuation of the Q wave which is often seen after the topical application of nucleotide.

A. Guinea-pig 83/48. Upper record before, lower record after the topical injection of 0·6 mg. MgATP in 0·03 ml. Lead III.

B. Guinea-pig 61/48. Upper record before, lower record after the topical injection of 0·6 mg. NaATP in 0·03 ml. Lead III.

Less importance was attached to the other changes which occurred in the shape of the ECG. Notable amongst these was accentuation of the Q wave (Fig. 3). On occasion this was so great that the R wave began and finished below the isoelectric level. Variable changes occurred in the T wave. In many experiments it was unchanged, whilst in others it was enlarged and sometimes reversed. It was not always in the same direction as the displaced ST segment.

These results refer to experiments in which ATP was injected into the surface layers of the left venticle. Similar injections into the right ventricle were technically difficult, but in successful experiments similar displacement of the ST segment was observed (Fig. 4). The topical application of ATP by the filter-paper method of Kisch, Nahum and Hoff (1940) also gave displacement of the ST segment (Fig. 5), but when ATP was given in this way there was no interference with AV conduction. In all these experiments the Na and Mg salts of ATP were equally effective.

Species other than the guinea-pig were tested. The injection of MgATP into the surface layers of the left ventricular muscle of the rabbit (0·8–2·0 mg.; 0·04–0·10 ml. solution) and cat (1·6–3·0 mg.; 0·08–0·15 ml. solution) produced

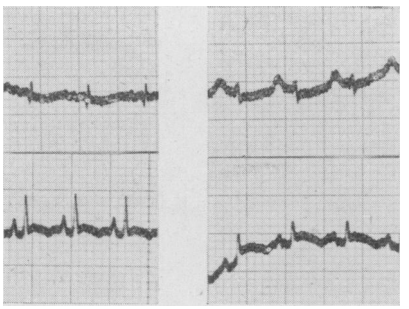

FIG. 4.—Effect of the injection of adenosine triphosphate into the surface of the right ventricle on the ECG of the guinea-pig under nembutal anaesthesia.

A = before;
B = after the topical injection of 0·2 mg. MgATP in 0·01 ml.

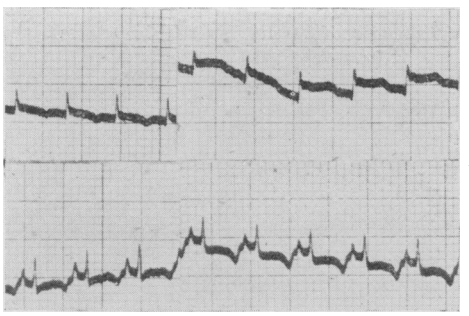

FIG. 5.—Effect of the topical application of adenosine triphosphate to the surface of the left ventricle by the filter-paper technique on the ECG of the guinea-pig under nembutal anaesthesia.

A = before;
B = after the application. Filter-paper approx. 5 × 5 mm. soaked in ATP solution 20 mg. per ml.

similar changes in the ST segment (Fig. 6). Here, although slight cardiac slowing occurred after the injection, there was no heart block or ventricular asystole. Simultaneous recording of the blood pressure in the cat showed that the topical application of ATP was accompanied by a fall in pressure, but the changes in the ST segment bore no relation to this fall. Nor did changes of the same extent occur when ATP was given intravenously in doses that caused an equal fall in blood pressure.

Both the injection of ATP into the heart wall of the toad and its successive application by the filter-paper technique produced displacement of the ST segment

(Fig. 7). These changes were slow to appear and slow to disappear and, in view of some results to be described later, are difficult to interpret.

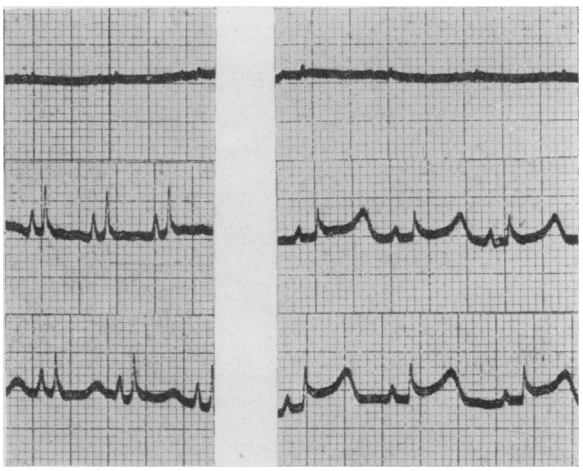

FIG. 6.—Effect of the injection of adenosine triphosphate into the surface of the left ventricle on the ECG of the cat under nembutal anaesthesia.

A = before;
B = after the topical injection of 3·0 mg. MgATP in 0·15 ml.

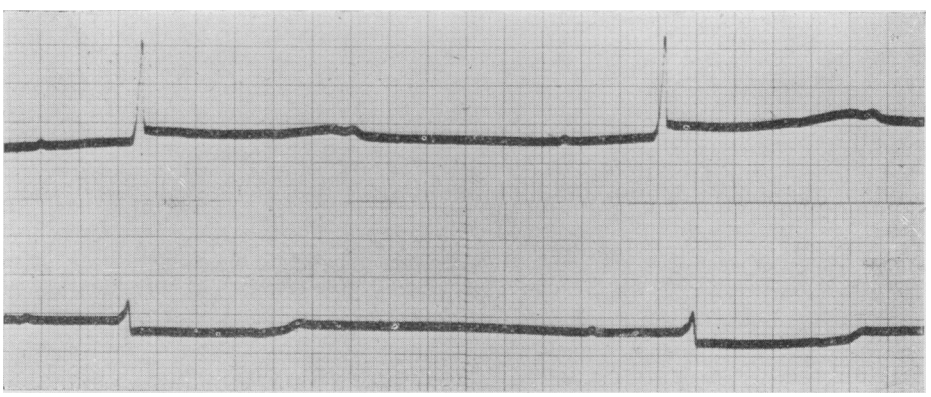

FIG. 7.—Effect of the injection of adenosine triphosphate into the ventricular wall on the ECG of the pithed toad. Lead III.

Upper record = before;
Lower record = after the topical injection of 0·6 mg. MgATP in 0·03 ml.

The effect of various substances given intravenously on the changes produced by topical application of ATP were also observed. The most important of these in view of its great significance in nucleotide metabolism was magnesium. When $MgSO_4$ (6–15 ml. 0.154 M solution per kg. body weight) was injected it produced sinus bradycardia and accentuation of the T waves, which were

sometimes inverted. Intraventricular block occurred when the doses were given rapidly. When given in doses of 10 ml. per kg. body weight it was found that whilst it prolonged the disturbance of rhythm after topical ATP, it decreased and usually prevented the appearance of the changes in the ST segment (Fig. 8).

Intravenous atropine sulphate (2·4 mg. per kg. body weight) had no effect on the ST segment changes after topical ATP. Theophylline ethylene diamine in doses up to 30 mg. per kg. body weight was given intravenously to control

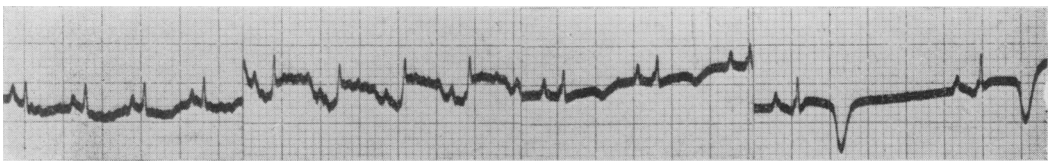

FIG. 8.—Effect of the intravenous administration of $MgSO_4$ on the response to the topical injection of adenosine triphosphate.

Guinea-pig 57/48. Lead III.

A = before;
B = after the topical injection of 0·8 mg. MgATP in 0·04 ml.
C = after the intravenous administration of 7·4 ml. 0·154 M $MgSO_4$ per kg. body weight.
D = after C, the topical injection of 0·8 mg. MgATP in 0·04 ml.

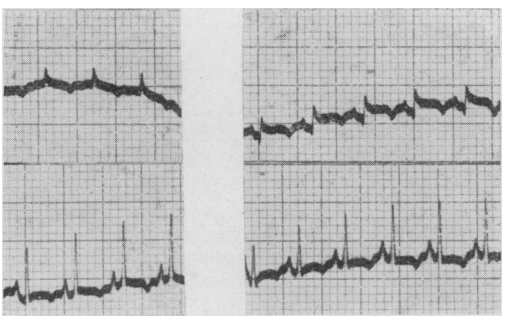

FIG. 9.—Effect of the injection of adenosine diphosphate into the surface of the left ventricle on the ECG of the guinea-pig under nembutal anaesthesia.

A = before;
B = after the topical injection of 0·6 mg. NaADP in 0·03 ml.

the effect of the coronary vasodilator properties of $MgSO_4$. After the coronary vessels had been widely dilated by this drug, ATP still produced the same alteration in the ST segment, although the period of irregular rhythm was reduced.

The topical application of ADP to the guinea-pig heart had exactly the same effect as ATP, both on the rhythm and on the form of the ECG, in which the displacement of the ST segment (Fig. 9) was just as obvious. ADP was slightly less active than ATP, so that a dose of 0·6 mg. NaADP gave an effect equivalent to that after 0·4 mg. NaATP. Intravenous $MgSO_4$ prevented the appearance of the ST displacement after topical ADP, as in the case of ATP. The removal of another phosphate group from the nucleotide molecule resulted in a further

loss in activity, for although A5MP, when injected in equimolecular amounts, would reproduce the disturbance in AV conduction seen after ATP, it had no effect on the ST segment. Much larger doses (0·8 mg.) were required to produce slight displacement of the ST segment, and the only constant change after topical A5MP was accentuation of the Q wave (Fig. 10). Adenosine, in doses up to 0·5 mg., had the same effect as the higher members of the series on the conducting system, but did not alter the shape of the ECG. The deaminated compound, ITP, in doses up to 0·9 mg., did not affect either the cardiac rhythm or the form of the ECG.

In order to exclude the possibility that these effects of the nucleotides were secondary to coronary vasoconstriction in the guinea-pig, we confirmed by perfusion experiments that these nucleotides had, as was expected, a dilator effect.

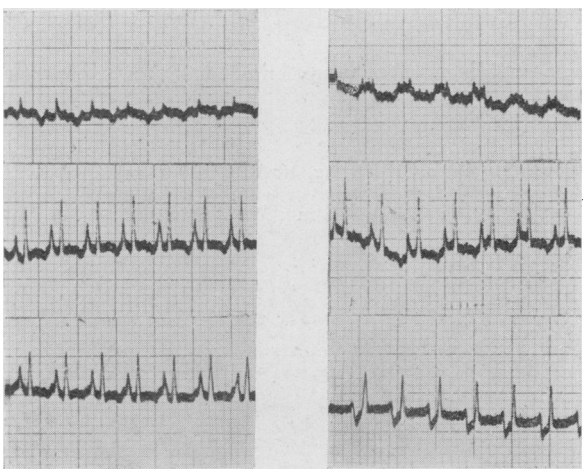

FIG. 10.—Effect of the injection of muscle adenylic acid into the surface of the left ventricle on the ECG of the guinea-pig under nembutal anaesthesia.

A = before;
B = after the topical injection of 0·6 mg. NaA5MP in 0·03 ml. (Dose in terms of free acid).

Topical application of other organic compounds.

The effect of the topical application to the heart of several other compounds which might possibly be concerned in the reactions of ischaemic tissue was also studied.

The pyrimidine nucleotide, cytidylic acid, known to be present in cardiac muscle (Drury, 1932), was tested in doses up to 0·8 mg., but had no effect on the form or rate of the ECG. Negative results were also obtained with injections of Na lactate as a 0·153 M solution in doses up to 0·8 mg. The experiments with Na lactate were, however, of interest, because intense coronary vasodilatation followed the topical application of this compound.

The effect of histamine was observed because, since the work of Lewis (1927), its possible release by damaged tissue has always remained a possibility. Anrep, Barsoum and Talaat (1936) claimed to have demonstrated the liberation of hista-

mine from the cardiac muscle of the dog during anoxaemia, but this claim could not be substantiated by Code, Evans and Gregory (1938).

Histamine acid phosphate, in doses of 20–40 μg., produced a very definite alteration in the form of the ECG (Fig. 11). These changes, deviation of the ST

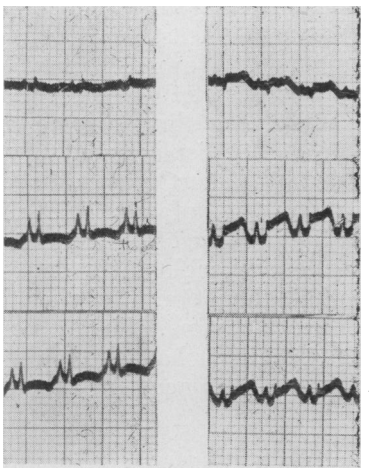

FIG. 11.—Effect of the injection of histamine into the surface of the left ventricle on the ECG of the guinea-pig under nembutal anaesthesia.

A = before ;
B = after the topical injection of 20 μg. histamine acid phosphate in 0·01 ml.

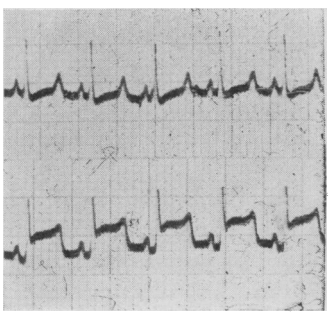

FIG. 12.—Effect of the injection of acetylcholine into the surface of the left ventricle on the ECG of the guinea-pig under nembutal anaesthesia. Lead III.

Upper record = before ;
Lower record = after the topical injection of 10 μg. acetylcholine hydrochloride in 0·01 ml.

segment and accentuation of the Q wave, appeared about 6 seconds after the injection, and closely resembled the changes produced by nucleotide. Similar changes have been observed by Koenigsfeld and Oppenheimer (1922) during anaphylactic shock in the guinea-pig. We found that the local effect of histamine

on the form of the ECG could be prevented by the previous intravenous injection of pyranisamine maleate ("Anthisan") in doses of 1·4–2·4 mg. per kg. body weight. It is very probable that the effect of histamine on the ECG is secondary to the coronary vasoconstriction which it produces in the guinea-pig (Gaddum, 1936). We confirmed this action on the perfused guinea-pig heart, and found that it could be antagonized by pyranisamine maleate.

The effect of acetylcholine on the heart bears certain superficial resemblances to that of the adenyl compounds. However, when acetylcholine was injected into the surface of the heart in doses of 10–60 μg. the results were very variable. The smallest effective dose was 10 μg., but in some animals even 60 μg. had no effect. An effective dose produced displacement of the ST segment (Fig. 12), which was frequently preceded by a short period of heart block. This latter was very variable, and the gross disturbance of ventricular rhythm seen after the topical injection of ATP was never reproduced by acetylcholine. Moreover atropine sulphate (1·0–2·7 mg. per kg. body weight intravenously) prevented the appearance of both heart block and ST deviation after the topical application of a previously effective dose of acetylcholine. Intravenous doses of prostigmine (0·077–1·54 mg. per kg. body weight) had the opposite effect, increasing both the period of heart block and the displacement of the ST segment after topical acetylcholine.

Topical application of inorganic compounds.

Many experiments were also performed in which solutions of inorganic salts were injected into the superficial layers of the myocardium. A variety of reasons determined the choice of compound. Solutions of NaCl were used to control the effect due to the presence merely of a volume of fluid in the myocardium, and also to determine the permissible limits of variation in tonicity of injected fluids. KCl was given because it has been shown by Dennis and Moore (1938) that ischaemia leads to the liberation of K^+ from cardiac muscle. $CaCl_2$ and $MgSO_4$ solutions were used, as these cations are cell constituents which may be concerned in the local response to injury. $Na_4P_2O_7$ and Na_2HPO_4 solutions were used to investigate the possible role of the phosphate fraction of the nucleotide molecule in the ATP response.

Sodium chloride.—In testing the influence of the volume of the solution, 0·154 M NaCl was injected into the superficial layers of the guinea-pig ventricle in doses up to 0·10 ml., the maximum volume of fluid used in that species. There was no change in the ST segment. Similar injections of 0·03 ml. 0·12 M NaCl in the toad, however, were not without effect, and produced deviation of the ST segment in a similar way to ATP. Possible reasons for this will be considered later. The application of NaCl solutions of appropriate molarity by the filter-paper technique was without effect in both the toad and the guinea-pig.

Whilst all the compounds were given, as far as possible, in iso-osmotic solutions, this could not always be done accurately. It was therefore essential to determine the range of tonicity in which solutions could be applied to the heart without altering the level of the ST segment. A constant volume (0·03 ml.) of NaCl solution of molarity varying between 0·616 and 0·038 was used. NaCl solutions in the range 0·308–0·103 M had no effect on the ST segment, but solutions of greater (0·616 M) or less (0·077 M) tonicity caused displacement. The displacement

observed was never as great as that seen in many experiments with ATP and K^+.

Potassium chloride.—A great deal is known of the action of K^+ on the form of the ECG. KCl displaces the ST segment on both topical and intravenous administration (Wiggers, 1929 ; Winkler, Hoff and Smith 1938), and Rothschuh (1939), who investigated the topical action of fresh extracts of cardiac and voluntary muscle, attributed the alterations he observed in the ST segment to the presence of K^+ in his extracts. Recently this action of K^+ has been studied in detail in the development of the view that electrical changes on the surface of the heart are important in determining the final form of the ECG (Kisch, 1940*a*, *b* ; Kisch, Nahum and Hoff, 1940), and in attempts to interpret normal and abnormal tracings (Hoff and Nahum, 1940 ; Hoff, Nahum and Kisch, 1941 ; Nahum, Hoff and Kisch, 1941 ; Nahum, Hamilton and Hoff, 1942 ; Robb, Dooley and Robb, 1942).

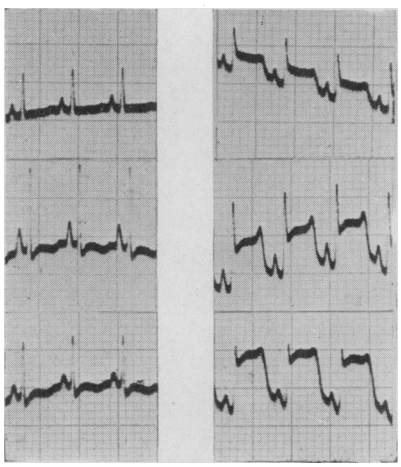

FIG. 13.—Effect of the injection of KCl into the surface of the left ventricle on the ECG of the guinea-pig under nembutal anaesthesia.

A = before ;
B = after the topical injection of 0·03 ml. 0·15 M KCl.

We also found that the topical application of KCl by either of the two methods produced a striking displacement of the ST segment (Fig. 13). The smallest effective dose was 0·02 ml. of 0·15 M KCl, provided that it was injected into the superficial layers of the myocardium. When the KCl solution was injected deeply into the muscle little change in the ECG was observed, confirming the work of Kisch, Nahum and Hoff (1940). The KCl injection produced no change in ventricular rate, and the ST alteration occurred immediately. With injections into the area shown in Fig. 1 the nature of the ST change was much more constant in type than after similar injections of ATP. In the 9 guinea-pigs used the ST segment was always elevated in Lead I and either depressed or elevated in Lead III. Potentiation of the Q wave was not seen. The T wave was always in the same direction as the displaced ST segment, all of which gave much more uniform curves than after ATP.

Atropine sulphate (1·0–2·7 mg. per kg. body weight intravenously) had no effect on the KCl response, nor did 0·154 M $MgSO_4$ (4–10 ml. per kg. body weight intravenously).

Calcium chloride.—Kisch (1940*a*) has shown that the topical application of $CaCl_2$ also produces displacement of the ST segment, and this was confirmed in

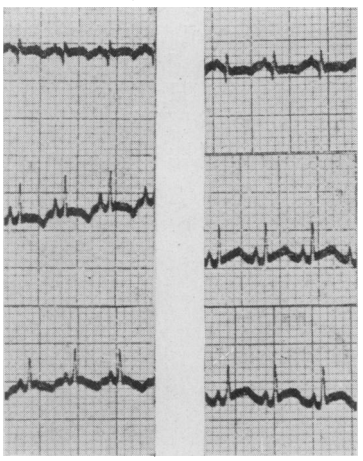

FIG. 14.—Effect of the injection of $CaCl_2$ into the surface of the left ventricle on the ECG of the guinea-pig under nembutal anaesthesia.
A = before;
B = after the topical injection of 0·04 ml. 0·10 M $CaCl_2$.

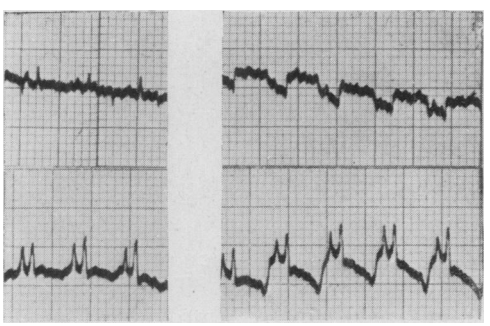

FIG. 15.—Effect of the injection of $Na_4P_2O_7$ into the surface of the left ventricle on the ECG of the guinea-pig under nembutal anaesthesia.
A = before;
B = after the topical injection of 0·03 ml. 0·1 M neutral $Na_4P_2O_7$.

experiments in which 0·04 ml. of 0·10 M $CaCl_2$ was injected (Fig. 14). The type of change closely resembled that produced by KCl, except that $CaCl_2$ did not appear to be so active. The intravenous injection of 0·154 M $MgSO_4$ (5–7 ml. per kg. body weight) had no effect on the response.

Magnesium sulphate.—In agreement with Kisch, Nahum and Hoff (1940), it

was found that the topical injection of 0·02–0·05 ml. of 0·154 M $MgSO_4$ had no effect on the level of the ST segment.

Sodium pyrophosphate.—When $Na_4P_2O_7$ was injected as a neutral 0·1 M solution in amounts equivalent in P_2O_7 content to effective doses of ATP no change was observed in the ST segment. However, increasing the dose produced displacement of the ST segment (Fig. 15). The smallest dose giving a constant effect was 0·03 ml. (0·522 mg. P_2O_7), which corresponds to 1·7 mg. NaATP. The picture produced was similar to that seen after ATP, but there was of course no change in heart rate. In interpreting these results one should appreciate that $Na_4P_2O_7$ constricts the coronary vessels in the guinea-pig.

Intravenous $MgSO_4$ in doses up to 7·0 ml. of 0·154 M $MgSO_4$ per kg. body weight did not affect the response, but with larger doses of 13·5 ml. a small reduction in the response did occur.

Sodium phosphate.—When Na_2HPO_4 was injected into the superficial layers of the myocardium constant effects were not obtained until 0·1 ml. of a neutral

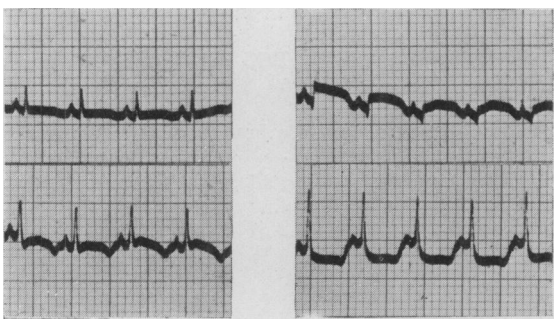

FIG. 16.—Effect of the injection of Na_2HPO_4 into the surface of the left ventricle on the ECG of the guinea-pig under nembutal anaesthesia.

A = before;
B = after the topical injection of 0·1 ml. 0·1 M neutral Na_2HPO_4.

0·10 M solution was reached. With this dose alterations in the ST segment occurred (Fig. 16). The amount of phosphate contained in this dose equals the amount which would be liberated by the decomposition of 2·52 mg. ATP to A5MP. The effect was still present after the intravenous injection of 7–11 ml. of 0·154 M $MgSO_4$ per kg. body weight.

Acute coronary occlusion.

The ECG changes after ligature of the coronary vessels supplying the left ventricle at the site shown in Fig. 1 were studied in 19 guinea-pigs. Fairly constant alterations were found (Fig. 17), the most important being in Lead III, where the ST segment was depressed in all except one animal. The ST segment was usually depressed in Lead II and elevated in Lead I. The Q wave was slightly accentuated in 4 animals, and the T wave was always in the same direction as the main ST deviation. The changes produced by ligature are therefore of the same order as those produced by the injection of active compounds in the area supplied by the artery.

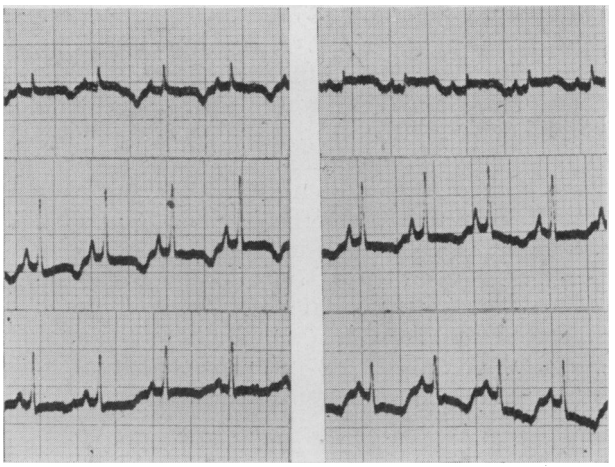

FIG. 17.—Effect of coronary occlusion on the ECG of the guinea-pig under nembutal anaesthesia.

Guinea-pig 17/48. A = before;
B = after ligature of a branch of left coronary artery at the site shown in Fig. 1.

This record should be compared with Fig. 2 D, as the coronary artery was ligated after the animal had recovered from the effects of the topical injection of ATP shown in that figure.

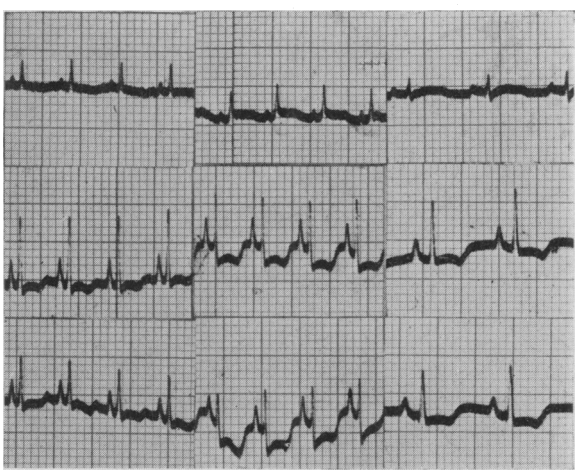

FIG. 18.—Effect of the intravenous administration of $MgSO_4$ on the ECG changes after ligature of a branch of the left coronary artery in the guinea-pig under nembutal anaesthesia.

A = before;
B = after ligature of a branch of the left coronary artery;
C = same after the intravenous injection of 17 ml. 0·154 M $MgSO_4$ per kg. body weight.

Having demonstrated this similarity we were chiefly interested in determining the effect on these changes of the various substances which had been found to affect the response to the topical application of the different compounds.

Atropine sulphate (1·0–2·7 mg. per kg. body weight intravenously) had no effect on the response to ligature, nor did prostigmine (<0·1 mg. per kg. body weight intravenously). Pyranisamine maleate (<4·3 mg. per kg. body weight) also had no effect. The inability of these compounds to alter the ECG changes of acute coronary occlusion was independent of whether they were given before or after ligature of the vessel.

In great contrast, intravenous $MgSO_4$ had a very definite effect on the ECG changes of coronary occlusion. When given in doses of 5–13 ml. of 0·154 M solution after ligature of the coronary vessels, its effect was to restore the ST segment to the isoelectric level either completely or partially, depending on the degree of the initial deviation (Fig. 18). $MgSO_4$ is a coronary vasodilator, and in order to eliminate this as a possible explanation for the effect the action of theophylline ethylene diamine was also investigated. In doses up to 30 mg. per kg. body weight intravenously this drug produced gross coronary vasodilatation, but had no significant effect on the displacement of the ST segment produced by ligature of the vessels ; if the ECG change after ischaemia were slight, reversal of the T wave was observed, as described by Bayley, La Due and York (1944), but that was the only effect observed. Of the various substances tested, therefore, $MgSO_4$ was the only one capable of reducing the current of injury produced in cardiac ischaemia. Since this cannot be accounted for by such non-specific effects as vasodilatation, it may be of the nature of a specific interference.

*Effect on Potential Difference across Isolated Muscle.**

Experiments were undertaken to determine directly the effects of ATP, A5MP, pyrophosphate, orthophosphate and adenosine on the potential difference (p.d.) across isolated membranes of voluntary muscle (rat diaphragm) and heart muscle (guinea-pig atrium).

The apparatus and technique used were similar to those described by Crane, Davies and Longmuir (1948). Sheets of rat diaphragm or guinea-pig atrium were mounted in perspex holders and placed between two glass chambers which were both filled with Krebs-Henseleit Ringer's solution containing 0·2 per cent glucose. The temperature was 38° C. The p.d. was measured on a sensitive galvanometer in a high resistance circuit by means of saturated calomel electrodes connected to the two chambers by saturated KCl bridges. The electrical resistance of the membrane was found by passing an electric current (of up to 1 mA./cm.^{2}) through it and measuring the p.d. drop. An allowance was made for the resistance of the saline medium.

The results are given in Table VI. Since changes in hydrostatic pressure of a few cm. water on either side of the membrane could produce a p.d. of about 1 mV. care was taken to keep constant the liquid levels in both chambers of the apparatus.

The electrical resistance of 8 sheets of diaphragm was from 34 Ω–cm^2 to 69 Ω–cm^2, average 52 Ω–cm^2, σ 11, and of 2 sheets of guinea-pig atrium was 112 and 133 Ω–cm^2. In no case did significant changes in the resistance of the tissues occur after the addition of the above substances.

* These experiments were carried out in collaboration with Mr. R. E. Davies.

TABLE VI.—*P.d. Changes Produced by Applying ATP, A5MP, Adenosine and Pyrophosphate and Phosphate Ions in Neutral Isotonic Solutions to One Side of a Membrane of Rat Diaphragm or Guinea-pig Atrium. In all cases there was no natural p.d. and the changes made the side in contact with the ATP, etc., negative to the unaltered side in an external circuit. The p.d. remained at 0·0 mV when equal volumes of isotonic NaCl were added to the medium. Readings accurate to 0·1 mV.*

Membrane.	Addition.	Concentration (M).	Potential difference produced (mV.).
Rat diaphragm	ATP	$2{\cdot}5 \times 10^{-3}$	1·3
	$Na_4P_2O_7$ (neutral)	$6{\cdot}0 \times 10^{-3}$	1·5
	Phosphate buffer pH 7·4	$6{\cdot}0 \times 10^{-3}$	1·1
Rat diaphragm	ATP	$2{\cdot}0 \times 10^{-3}$	0·45
	Phosphate buffer pH 7·4	$3{\cdot}0 \times 10^{-3}$	0·25
	Adenosine	$2{\cdot}0 \times 10^{-3}$	0·0
	Adenosine	$4{\cdot}0 \times 10^{-3}$	0·0
	ATP	$2{\cdot}0 \times 10^{-3}$	0·45
Rat diaphragm	A5MP	$2{\cdot}5 \times 10^{-3}$	0·85
	Phosphate buffer pH 7·4	$7{\cdot}5 \times 10^{-3}$	0·95
Guinea-pig atrium	Adenosine	$2{\cdot}5 \times 10^{-3}$	0·0
	ATP	$2{\cdot}5 \times 10^{-3}$	0·47
Guinea-pig atrium	ATP	$0{\cdot}8 \times 10^{-3}$	0·5
	ATP	$2{\cdot}0 \times 10^{-3}$	1·7
	ATP	$2{\cdot}0 \times 10^{-3}$	1·1

The estimations of p.d. showed that phosphate ions given in the inorganic form or in the form of the nucleotide can produce changes in p.d. of the order of 1 mV. when applied in concentrations of 1 to 10 mM to one side of a membrane of voluntary or cardiac muscle. Adenosine was without effect. This supports the view put forward above in explanation of the effects of nucleotides, applied topically to the heart, on the level of the ST segment.

The effect of nucleotide and the inorganic ions on the level of the ST segment appears to be due to a specific effect on the electrical properties of the cardiac muscle. The inorganic ions are thought to alter the distribution of the charges about the cell membrane and so establishing a potential difference. In the case of the adenine nucleotides it is reasoned that this occurs as a result of the liberation of phosphate groups in its enzymic decomposition. Mg^{++} is known to inhibit the dephosphorylation of these compounds and $MgSO_4$ administration prevents the effect of the nucleotides whilst leaving that of the inorganic ions unchanged. These results show that the application of ATP and phosphates but not adenosine to one surface of a muscle sheet gives rise to a potential difference across the muscle.

The effect of coronary occlusion on the level of the ST segment is unaltered by the administration of atropine, prostigmine and pyranisamine maleate, but $MgSO_4$ restores the segment to the isoelectric level. This suggests that the free phosphate groups formed in the anoxaemic breakdown of nucleotide play a part in the production of the abnormal ECG of coronary occlusion.

DISCUSSION.

The results of these experiments bear on both the chemical and electrical changes which occur in ischaemic cardiac muscle. The chemical determinations show that after occlusion of a coronary artery the nucleotide content of the muscle supplied by that vessel falls. This is due to the dephosphorylation of the ATP initially present. This is in agreement with the results of Burns and Cruickshank (1937), and of Chang (1938), who studied the distribution of phosphate in the heart after alterations in the O_2 content of the perfusion fluid. Dephosphorylation of ATP under anoxaemic conditions is to be expected, and corresponds closely to that which occurs when the heart muscle is allowed to autolyse at 37° C. for the same period of time. The actual degree of dephosphorylation which occurs in these two conditions is probably greater than that shown in the results because, owing to insufficient material, we were unable to correct the "7 minute" P figure for the percentage of the "60 minute" P value liberated by the shorter hydrolysis. However, the changes in nucleotide in these two conditions are not entirely similar, for during autolysis deamination of the nucleotide molecule also occurs. Deamination does not occur to this extent when one coronary artery is occluded. It is improbable that the occlusion of one coronary artery will render the muscle in its area of supply completely ischaemic. Although insufficient dye enters to cause visible staining, a small trickle of perfusing fluid probably passes through this muscle from the collateral circulation provided by the other artery. The small amount of O_2 which reaches the muscle in this way is probably sufficient to prevent deamination, as Macfarlane and Spooner (1946) have shown in voluntary muscle that extreme conditions of anoxia, such as do occur in autolysis, are required before deamination of nucleotide occurs. Despite these changes during the 1-hour period of anoxaemia there is no evidence that either nucleotide, or its breakdown products, leave the muscle during this time. The experiments in which ATP was injected within the ischaemic area showed that if any nucleotide did become extracellular under these conditions it would be possible for it to leave the heart.

With the restoration of the circulation some rejuvenation of the degraded nucleotide occurs and the ATP content of the muscle increases. The significance of the low adenosine and total pentose values at this time is obscure, but with the absence of any change in total P together with no evidence of adenosine in the perfusate, liberation of nucleotide from the heart during the first 30 minutes after a 1-hour period of anoxaemia would not appear to occur. This does not mean that anoxaemia cannot lead to the liberation of nucleotide and/or its breakdown products from cardiac muscle, but simply that no such effect occurs under our experimental conditions. In other experiments on voluntary muscle, where evidence in favour of nucleotide release was obtained, the period of ischaemia was much longer (4 hours), and the state of the muscle after restoration of the circulation was followed for a much longer time. Comparable experiments on the heart, at similar temperature ranges, will be necessary before the possibility can be excluded. Furthermore, although adenyl compounds do not appear to be liberated into the extracellular spaces by short periods of anoxaemia, they may still participate in the electrical changes resulting from that injury.

The topical application to the heart of ATP and certain other substances has a remarkable effect in causing displacement of the ST segment, and so repro-

ducing quite closely the most important ECG change of acute coronary occlusion. This resemblance is not only confined to the form of the ECG ; in both cases it is the heart surface that must be affected before any ECG change is produced. On the other hand, the correlation between the site of the injection and the direction of the changes in the three leads is not so constant as it is after the ligature of branches of the coronary arteries. This was also noted by Korey and Katz (1934) in experiments on chemical injuries to the myocardium, and is probably due to a greater surface area being involved after coronary occlusion.

The ECG changes may be due to the specific action of the active compounds on the electrical properties of cardiac muscle, or to a non-specific indirect mechanism such as coronary vasoconstriction. The effect of histamine and acetylcholine is in fact most probably due to their constrictor action on the coronary vessels of the guinea-pig. Many of the active compounds, however, either dilate or have no action upon the coronary vessels, but there are other non-specific ways in which the effect might be produced.

The possibility that distension of the myocardium by the volume of fluid injected is responsible for the effect was excluded by finding no response after injecting equal volumes of isotonic NaCl. The compounds are also active when applied by the filter-paper technique where this possibility does not arise. In the toad the injection of isotonic NaCl did displace the ST segment, but the structure of the heart wall in this species is so different from that in the mammal that the two experiments are not comparable. Moreover isotonic saline applied in the toad by the filter-paper technique has no effect.

Most of the substances were applied in isotonic solutions, but the possible effect of small variations in the tonicity of some solutions was excluded by showing that quite a wide range of variation was permissible.

The topical application of nucleotide lowers the blood pressure and alters both the rate and shape of the heart. All of them can, as factors in the ECG changes, however, be excluded. We have seen in the cat that there is no relationship between the fall in the blood pressure and the displacement of the ST segment. Equimolecular amounts of adenosine give the same changes in heart rate as ATP when injected in the guinea-pig, yet adenosine does not alter the level of the ST segment. In the cat and rabbit ATP produces very little change in heart rate, yet the ST segment is displaced. Nor do major changes in heart rate occur after the application of ATP by the filter-paper method, though this is a satisfactory method for inducing ECG changes. The fact that displacement of the ST segment can be produced in the toad heart shows that it is not dependent upon the presence of a conducting system. The changed shape of the heart is secondary to the changed heart rate, and furthermore, Buchbinder and Katz (1934) have shown by direct experiment that a change of this type does not of itself cause any alteration in the ST segment.

The above arguments concern the exclusion of non-specific factors in the response to nucleotide in particular. Similar arguments may be used in connection with the mechanism of action of the inorganic ions. It would seem, therefore, that the changes in the ST segment produced by adenine nucleotide and certain inorganic ions are due to a specific effect on the electrical properties of the cardiac muscle.

We have found that the direct effect of adenine nucleotide on cardiac muscle is related to the liberation of the phosphate group (Green and Stoner, 1949).

The significance of the finding that the change in the ST segment can be positively correlated with the phosphate content of the nucleotide, in that A5MP is less active than ADP, which, in turn, is less active than ATP, becomes apparent. Moreover the effect can be reproduced by inorganic pyro- and orthophosphate. Admittedly the amounts of inorganic phosphate used are greater than the equimolecular requirements, but this might be expected on the basis that the organic phosphates will be biologically more acceptable. The hypothesis proposed for the mode of action of adenine nucleotides in altering the ECG is as follows: After topical application enzymic dephosphorylation occurs with the liberation of phosphate groups. These phosphate groups alter the distribution of the charges about the cell membrane, producing a current of injury which alters the level of the ST segment. Support for this theory is to be found in two pieces of evidence. It is known that Mg^{++} inhibits the dephosphorylation of ATP by tissues *in vitro* (Banga and Szent-Györgyi, 1943; Stoner and Green, 1945*b*), and we have also found that Mg^{++} inhibits the direct action of ATP on the contraction of cardiac muscle. Therefore, our finding that Mg^{++} administration prevents the appearance of the changes in the ST segment after the topical application of nucleotide supports the view that these changes are due to the liberation of phosphate groups. This action of Mg^{++} cannot be accounted for by its non-specific effects on the heart, i.e. coronary vasodilatation and bradycardia. Doses of theophylline ethylene diamine which cause gross coronary vasodilatation have no effect on the ST changes after topical ATP. Intravenous $MgSO_4$ does not affect the response to inorganic ions (e.g. K^+). The absence of any significant interference by Mg^{++} in the response to inorganic phosphate also supports the view that nucleotide acts through the liberation of phosphate groups, and that this process is inhibited by Mg^{++}.

Favourable evidence of a more direct nature has also been obtained from the experiments showing that the application of adenine nucleotide to one surface of a sheet of muscle (voluntary or cardiac) leads to the development of a potential difference across the muscle of about the order of magnitude anticipated from the ECG experiments. This effect of nucleotide could be reproduced by pyro- and orthophosphate, but adenosine was without effect. These results fit in very well with our hypothesis for the action of adenine nucleotide on the level of the ST segment.

With regard to the activity of the other inorganic ions such as K^+ and Ca^{++}, it is reasonable to suppose that they may act in a similar way by altering the balance of the charges about the cell membrane.

The ultimate and practical question is whether all these findings throw any light on the mechanism of the ECG changes of acute coronary occlusion. Displacement of the ST segment is produced by four substances which might be involved in the response to muscle ischaemia, namely nucleotide, histamine, acetylcholine and K^+. In the case of histamine and acetylcholine the amounts required to produce the effect are probably larger than those which could be involved in the ischaemic response. But more important, their effects are antagonized by the intravenous administration of pyranisamine maleate and atropine respectively, and these drugs have no effect on the changes in the ST segment after coronary occlusion. On the other hand, the amounts of nucleotide and K^+ which are required approximate to those which might be involved in the damaged muscle. It is at this point that the action of Mg^{++} becomes impor-

tant, for $MgSO_4$ administration prevents the changes in the ST segment after the topical application of nucleotide but not of K^+. $MgSO_4$ administration also restores the displaced ST segment after coronary occlusion to a more normal position, and this is not due to non-specific coronary vasodilatation, since theophylline ethylene diamine has not the same effect. The action of Mg^{++} therefore suggests that if either of these substances plays any part in the production of the ECG changes of coronary occlusion it is the nucleotide and not K^+. The idea that the displacement of the ST segment in acute coronary ischaemia is produced in this way is an attractive one. Indeed Burge, Orth, Neild, Krouse and Wickwire (1936) have suggested, on somewhat inadequate evidence, that phosphate groups may be concerned in the current of injury in frog muscle, and more recently Nachmansohn, Cox, Coates and Machado (1943) have suggested that the action potentials of the electric organ may be related to reactions involving phosphocreatine and ATP. Cardiac ischaemia is associated with the rupture of high energy phosphate bonds and the liberation of phosphate groups in amounts which are probably sufficient to account for the effect. The fact that in this case the phosphate groups are intracellular whereas in experiments on the topical application of ATP they are extracellular is probably not important, since in both cases the phosphate groups could alter the balance of the charges about the cell membrane and produce the effect on the ST segment. Whilst our results make it probable that the ultimate explanation of the changes in the ST segment in cardiac ischaemia will be along these lines, final proof of the theory may well be difficult to obtain.

SUMMARY.

The effect of occlusion of the left coronary artery on the distribution of nucleotide and phosphate in the myocardium of the left ventricle has been examined in the perfused rabbit heart.

During a 1-hour period of occlusion the fully phosphorylated nucleotide tends to break down to the lower members of the series without any loss of the component parts of the nucleotide molecule from the muscle. These changes are similar to those which take place during a 1-hour period of autolysis at 37° C., except that under the latter conditions deamination of the adenosine occurs. This is thought to imply that the degree of anoxia is very much greater during autolysis than after occlusion of the coronary artery.

On restoration of the circulation some rejuvenation of the degraded nucleotide occurs, and by the end of 30 minutes the " 7 minute " P level and the percentage of pentose in the phosphorylated form is greater than at the end of the period of anoxaemia. Again, there is no evidence for the release of any nucleotide or its breakdown products during this period.

The changes in the nucleotide which occur under our experimental conditions appear to take place within the muscle cells. There is no evidence that either fully formed nucleotide, or its breakdown products, passes into the extracellular spaces as a result of trauma of this type and duration. Nucleotide, experimentally injected into the extracellular space, does in fact pass into the perfusate.

The topical application of certain substances to the heart causes displacement of the ST segment similar to that produced by coronary occlusion.

The following substances give this effect in some degree : ATP, ADP, A5MP, acetylcholine, histamine, Na_2HPO_4, $Na_4P_2O_7$, K^+ and Ca^{++}. The following

are inactive : Adenosine, ITP, cytidylic acid, Na lactate, Mg^{++} and Na^{+}. These results refer to the guinea-pig. ATP has been found to be active in the cat, rabbit and toad.

The ECG effects of acetylcholine are antagonized by atropine and accentuated by prostigmine. The ECG effects of histamine are antagonized by pyranisamine maleate. The ECG effects of these substances are probably non-specific and secondary to their action on the coronary circulation of the guinea-pig.

The effect of coronary occlusion on the level of the ST segment is unaltered by atropine, prostigmine or pyranisamine maleate, but Mg^{++} restores the segment to the isoelectric level.

Mg^{++} antagonizes the effects on the ST segment of ATP and ADP, probably by inhibiting their dephosphorylation. The effects of K^{+}, Ca^{++} and Na_2HPO_4 are unaltered, and that of $Na_4P_2O_7$ only slightly reduced.

The application of adenine nucleotide to one surface of a sheet of muscle (voluntary or cardiac) produces a potential difference across the muscle of an order of magnitude which could account for the ECG changes. A similar effect is obtained with inorganic pyro- and orthophosphate, but not with adenosine.

On the basis of these and other findings discussed in the text, the following hypothesis is proposed. The displacement of the ST segment following coronary occlusion is due to the liberation of free phosphate groups formed in the anoxaemic breakdown of nucleotide. The alteration in the balance of charges about the cell membrane thus produced, displaces the ST segment from the isoelectric level.

We are deeply indebted to Mr. R. E. Davies for collaborating in the work on the potential differences across isolated muscle.

Our thanks are due to Prof. E. J. Wayne for his advice, to Mr. D. E. Hughes for his assistance with the spectrophotometry, and to Messrs. May & Baker, Ltd., for a gift of pyranisamine maleate ("Anthisan ").

The expenses of this work were defrayed by the Medical Research Council, and two of us (H.B.S. and C.J.T.) are in receipt of whole-time personal grants from this source.

REFERENCES.

ANREP, G. V., BARSOUM, G. S., AND TALAAT, M.—(1936) *J. Physiol.*, **86**, 431.
BANGA, I., AND SZENT GYÖRGYI, A.—(1943) *Studies from Inst. Med. Chem. Szeged*, **3**, 72.
BAYLEY, R. H., LA DUE, J. S., AND YORK, D. J.—(1944) *Amer. Heart J.*, **27**, 657.
BEATTIE, F., MILROY, T. H., AND STRAIN, R. W. M.—(1934) *Biochem. J.*, **28**, 84.
BENNET, D. W., AND DRURY, A. N.—(1931) *J. Physiol.*, **72**, 288.
BUCKBINDER, W. C., AND KATZ, L. N.—(1934) *Amer. J. med. Sci.*, **187**, 785.
BURGE, W. E., ORTH, O. S., NEILD, H. W., KROUSE, R., AND WICKWIRE, G. C.—(1936) *J. Lab. clin. Med.*, **21**, 1162.
BURNS, W., AND CRUICKSHANK, E. W. H.—(1937) *J. Physiol.*, **91**, 314.
CHANG, I.—(1938) *Quart. J. exp. Physiol.*, **28**, 1.
CODE, C. F., EVANS, C. L., AND GREGORY, R. A.—(1938) *J. Physiol.*, **92**, 344.
CRANE, E. E., DAVIES, R. E., AND LONGMUIR, N. M.—(1948) *Biochem. J.*, **43**, 121.
DAVIES, F., FRANCIS, E. T. B., AND STONER, H. B.—(1947) *J. Physiol.*, **106**, 154.
DENNIS, J., AND MOORE, R. M.—(1938) *Amer. J. Physiol.*, **123**, 443.
DRURY, A. N.—(1932) *J. Physiol.*, **76**, 15P.

Idem, LUTWAK-MANN, C., AND SOLANDT, O. M.—(1938) *Quart. J. exp. Physiol.*, **27**, 215.
GADDUM, J. H.—(1936) ' Gefässerweiternde stoffe der Gewebe.' Leipzig (Thieme).
GREEN, H. N., BIELSCHOWSKY, M., AND STONER, H. B.—(1947) Unpublished Report to Mes. Res. Counc., Lond.
Idem AND STONER, H. B.—(1949) 'Biological Actions of the Adenine Nucleotides.' London (H. K. Lewis). (In press.)
HOFF, H. E., AND NAHUM, L. H.—(1940) *Proc. Soc. exp. Biol., N.Y.*, **45**, 263.
Idem AND KISCH, B.—(1941) *Amer. J. Physiol.*, **131**, 700.
KATZ, L. N.—(1946) ' Electrocardiography.' London (H. Kimpton); (1947) *Physiol. Rev.*, **27**, 398.
KISCH, B.—(1940*a*) *Cardiologia*, **4**, 304; (1940*b*) *ibid.*, **4**, 318.
Idem, NAHUM, L. H., AND HOFF, H. E.—(1940) *Amer. Heart J.*, **20**, 174.
KOENIGSFELD, H., AND OPPENHEIMER, E.—(1922) *Z. ges. exp. Med.*, **28**, 106.
KOREY, H., AND KATZ, L. N.—(1934) *Amer. J. med. Sci.*, **188**, 387.
KREBS, H. A., AND HENSELEIT, K.—(1932) *Hoppe-Seyl. Z.*, **210**, 33.
LEWIS, T.—(1927) ' Blood Vessels of the Human Skin and their Responses.' London (Shaw).
MACFARLANE, M. G., AND SPOONER, S. J. L.—(1946) *Brit. J. exp. Path.*, **27**, 339.
MEJBAUM, W.—(1939) *Hoppe-Seyl. Z.*, **258**, 117.
NACHMANSOHN, D., COX, R. T., COATES, C. W., AND MACHADO, A. L.—(1943) *Proc. Soc. exp. Biol., N.Y.*, **52**, 97.
NAHUM, L. H., HOFF, H. E., AND KISCH, B.—(1941) *Amer. J. Physiol.*, **131**, 693.
Idem, HAMILTON, W. F., AND HOFF, H. E.—(1942) *ibid.*, **139**, 202.
PETERS, J. P., AND VAN SLYKE, D. D.—(1932) ' Quantitative Clinical Chemistry,' vol. ii, Baltimore (Williams & Wilkins).
ROBB, J. S., DOOLEY, M. S., AND ROBB, R. C.—(1942) *J. Mt. Sinai Hosp.*, **8**, 946.
ROTHSCHUH, K. E.—(1939) *Z. ges. exp. Med.*, **106**, 543.
STONER, H. B., AND GREEN, H. N.—(1944) *J. Path. Bact.*, **56**, 343; (1945*a*) *ibid.*, **57**, 337; (1945*b*) *Biochem. J.*, **39**, 474.
WAYNE, E. J., GOODWIN, J. G., AND STONER, H. B.—(1948). *British Heart J.* (In press.)
WIGGERS, C. J.—(1929) *Amer. Heart J.*, **5**, 346.
WINKLER, A. W., HOFF, H. E., AND SMITH, P. K.—(1938) *Amer. J. Physiol.*, **124**, 478.