4. As no interaction between the N-oxides and quinones occurred in the absence of the organisms, the quinones must have participated to some degree in the metabolism of the latter. The participation may be no more intimate than that of being concerned with hydrogen transport, but the structural similarity between the N-oxides and quinones suggests that the two classes of compounds may function at common sites in the organism, and that the action of iodinin may be to inhibit systems normally concerned with such quinones.

I am greatly indebted to Mr D. E. Hughes for assistance throughout these investigations.

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Factors Influencing the Nucleoprotein Content of Fibroblasts Growing *in vitro*

By J. N. DAVIDSON AND CHARITY WAYMOUTH, *From the Biochemical Laboratory, Physiology Department, The University of Aberdeen*

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Since Carrel [1912] first showed that the growth of tissue cultures *in vitro* could be stimulated by means of extracts of embryonic tissue, and that cultures could be maintained apparently indefinitely in such extracts, much speculation has arisen as to the chemical nature of the constituents of embryo juice responsible for the stimulation of cellular proliferation. Baker & Carrel [1926] came to the conclusion that activity lay in the protein fraction, and obtained active materials by precipitation of these with ethanol or CO_2. Rejecting the theory that embryo juice contained a specific hormone for cell proliferation, they later suggested [Carrel & Baker, 1926] that growth-activating substances are not preformed in embryo juice but are continuously made from its protein, perhaps in the cells of the cultures themselves, as the result of enzyme action. In support of this suggestion they pointed out that proteoses and higher protein degradation products have very potent growth-promoting properties, an observation which was confirmed and extended by Willmer & Kendal [1932]. Needham [1931] also rejected the view that any one particular factor was responsible for the growth-promoting properties of embryo juice, which he regarded as 'a special collocation of the right nutrient substances probably protein break-down products', but later [Needham, 1942] he did not exclude the participation of 'some carrier substance of nucleotide type'. However, Fischer [1939; 1940] prepared a very active growth-promoting factor from beef embryos by isolating the nucleoproteins. The activity seemed to be located in the nucleoprotein fraction containing the ribonucleic acids, but repeated reprecipitation of the nucleoproteins resulted in loss of activity. In the active fractions the phosphorus content was higher than could be accounted for by the nucleic acid content. Some active fractions contained sulphur, probably derived from chondroitin or mucoitin sulphuric acid complexes precipitated with the nucleoproteins. The presence of ribonucleic acids in rapidly growing tissues is now well recognized [Caspersson & Schultz, 1939; 1940; Mitchell, 1940; Schultz, 1941], and the nucleoproteins of several different embryonic tissues have been shown to contain ribonucleic acids [Davidson & Waymouth, unpublished results].

By the term 'growth', applied to cells *in vitro*, as distinct from mere survival of the cells with or without cell migration and even cell division, is

meant cellular proliferation with the formation of new protoplasm. A number of criteria of growth have been suggested, e.g. the area of the culture [Ebeling, 1921], the mitotic index [Willmer & Kendal, 1932], the mass of the culture [Meier, 1931; Laser, 1933*b*; Wilson, Jackson & Brues, 1942], its metabolic activity [Meier, 1931; Laser, 1932; 1933*a*; Lipmann, 1932; 1933] or the nucleoprotein content [Willmer, 1942] as determined by chemical estimation of the nucleoprotein P (N.P.P.). The last has appeared to us to be most satisfactory although, as Willmer [1942] points out, it suffers from the defect that nucleic acid or nucleoprotein is a cytoplasmic constituent in some tissues [cf. Caspersson & Schultz, 1940]. Moreover, its chemical determination is based on the assumption that when a tissue has been extracted with fat solvents and with dilute acid, the residual P is all present as nucleic acid. Some may be present as phosphoprotein P [Plimmer & Scott, 1908], but the amounts of this in most tissues, including embryonic tissue, is very low [v. Euler & Schmidt, 1934], or negligible [Masing, 1911].

The work of Brues, Tracy & Cohn [1942] with radioactive P, suggests that the above assumption may not be fully justified for all tissues, but in agreement with Willmer we have found that the estimation of the 'nucleic acid phosphorus' provides a satisfactory index of growth in the case of cultures from the embryo chick heart.

MATERIALS AND METHODS

As reported in a preliminary communication [Davidson & Waymouth, 1942] we have employed the general technique described by Willmer [1942], involving the use of constricted roller tubes made of Pyrex glass.

General. All the tests have been carried out with fresh explants from the heart of the 9-day chick embryo. No experiments have been carried out on cultures maintained as a pure strain. This must be kept in mind when considering the results. Plasma was obtained from young cockerels. The Tyrode solution used had the composition described by Willmer & Kendal [1932], and was sterilized by filtration through a Berkefeld candle. Chick embryo extract was prepared by pulping 9-day chick embryos in sterile graduated centrifuge tubes with an equal volume of Tyrode solution, freezing, thawing, centrifuging, and pipetting off the supernatant fluid into sterile storage tubes. This concentrated extract (total N = 40–60 mg. %) was suitably diluted before use.

Control experiments. As the composition of plasma is variable, direct comparisons of the effects of two substances on the tissue N.P.P. are made only where control and test series are grown in plasma from the same sample at the same time. For a similar reason, when embryo extract with and without some supplement is tested, comparisons are drawn only when a single sample of extract is used throughout. The effect of any lack of uniformity, due to inherent variations in potential growth capacity between different hearts, is reduced by using in each test pieces from a mixed sample from 6 to 8 hearts.

Preparation of explants. Each of the embryo hearts was cut up into 20–30 small and approximately uniform pieces according to the usual aseptic tissue culture technique [cf. Strangeways, 1924]. Six pieces of tissue were implanted in 0·2 ml. sterile plasma in each roller tube. The tubes were set aside until the plasma had clotted and 0·5 ml. Tyrode solution was then added. The tubes were stoppered with sterile rubber bungs, and mounted on a drum which rotated, by means of a small electric motor, in the incubator at 38° at a speed of 1 revolution/min. With each revolution the fluid washed over the growing cultures.

Six to eight of the tubes contained a plasma clot but no tissue, and served as plasma blanks. The remainder contained 6 pieces of tissue each. As the drum carried a total of 20 tubes, each test involved the use of some 70–80 pieces of tissue.

Technique of experiments. The tubes were rotated, with Tyrode solution alone as fluid phase, for 2 days to allow traces of growth-promoting substances present in the fresh tissue pieces to be used up, and to allow the cultures to be as uniform as possible before addition of test materials. The 'residual growth energy' is reported by Trowell & Willmer [1939] to be exhausted after this time. The tubes were then opened and the fluid phase (Tyrode + serum + metabolic products) sucked off. The test proper then began.

To each tube was added Tyrode, embryo extract, or test substance, or a combination of two or three of these, the total volume being 0·5 ml. As many duplicates as possible were set up. The tubes were re-stoppered and rotated for a further 3 hr. to allow the fluid to permeate the plasma clot. Some of the tubes were removed at this time (time = 0) to give figures for the initial value of N.P.P. The remainder were allowed to run for a further period of time, usually 2 days (time = t).

At 'time t' the tubes were removed. By means of a projectoscope, images of the cultures could be either outlined on paper and the areas subsequently determined with a planimeter, or recorded in silhouette on Ilford Reflex Document paper, although the curvature of the roller tubes made very accurate projection impossible.

Estimation of nucleic acid phosphorus. The method of Willmer [1942] was again followed. The roller tube became a test tube, and lipid P and acid-soluble P were extracted by ethanol-chloroform and 0·1 N HCl respectively, according to the procedure of Berenblum, Chain & Heatley [1939]. The tissue remained in the plasma clot throughout this process. It was then ashed in the roller tube with 0·15 ml. 70% perchloric acid, and P was determined by the ultra-micro-method of Berenblum & Chain [1938]. As originally described, the method is time consuming, since each estimation must be done individually, but we have devised a new type of mixing vessel (Fig. 1) which saves time by allowing

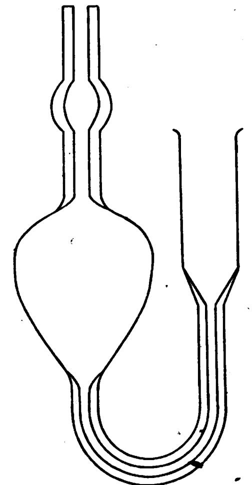

Fig. 1. Mixing vessel for phosphate estimation. $\frac{3}{4}$ actual size.

a large number of estimations to be done simultaneously. A battery of seven (or more) of these vessels is set up in a rack so that at least six estimations and one standard can be carried through at the same time. The reagents are pipetted into the cup on the short limb and are transferred to the bulb by gentle suction applied to the long limb. After mixture of the aqueous and butanol layers in the bulb, the aqueous layer settles out and can be sucked off through a capillary pipette inserted into the bottom of the cup. The blue alcoholic layer is finally poured from the cup into a small tube graduated at 1 ml., and the colour is read off either in a colorimeter against a standard prepared simultaneously, or in the Hilger Spekker Absorbtiometer with micro-cups.

The figure for the N.P.P. in the tissue alone is determined by subtracting the appropriate plasma blank from the N.P.P. figure for each tube containing tissue. The index $(100Y)/X$ gives an indication of the increase in N.P.P. relative to the amount originally present where X and Y are the figures for the N.P.P. at 'time 0' and 'time t' respectively.

RESULTS

Tyrode solution alone as fluid phase

In agreement with Willmer [1942] we find a drop in N.P.P. in the course of the experiment (Table 1). In 23 different experiments the average figure for the initial N.P.P. was 1·28 μg./roller tube (after subtraction of appropriate plasma blanks). After 2 days the N.P.P. had fallen to 1·13 μg. (Table 1). No significant alteration in N.P.P. occurred in the tubes containing plasma and Tyrode without tissue.

Table 1. *Changes in N.P.P. (nucleoprotein P) in roller tubes containing Tyrode solution alone as fluid phase*

All tubes run for 2 days with Tyrode solution alone before test begins. Fresh Tyrode then introduced (time 0) and initial N.P.P. measurements made. Final N.P.P. estimation carried out 2 days later (time t).

	Initial N.P.P. at 'time 0' per roller tube μg.	Final N.P.P. at 'time t' per roller tube μg.	Diff.	No. of exps.
Tubes with plasma alone	0·29 ± 0·01	0·31 ± 0·02	+0·02	26
Tubes with tissue (corrected for plasma blanks)	1·28 ± 0·07	1·13 ± 0·06	−0·15	23

It is probable that this fall is due to the presence in the plasma of nucleases which break up the nucleic acid in such of the cells as die in the course of the 2 days. We have demonstrated the presence of such nucleases in cockerel plasma and serum. A mixture of 0·2 ml. cockerel plasma with 0·3 ml. veronal-acetate buffer (pH 7·6) and 0·1 ml. 2% thymonucleic acid or ribonucleic acid (as Na salts) was incubated at 37°. At suitable intervals portions were treated with an equal volume of 0·25% uranyl acetate in 2·5% $CCl_3.COOH$. The precipitate of unhydrolysed nucleic acid was centrifuged down, washed with 0·125% uranyl acetate in 1·25% $CCl_3.COOH$, and dissolved in Na_2CO_3 solution. Total P was determined in this solution by a modification of the method of Allen [1940]. Some spontaneous decomposition of nucleic acids takes place at pH 7·6, which was allowed for by a control series without plasma. Both types of nucleic acid are hydrolysed by plasma to compounds no longer precipitable by the uranyl acetate reagent (Table 2), but the ribonucleic acid suffers more decomposition than the thymonucleic acid. Cockerel plasma therefore contains enzymes of the nuclease type such as ribonuclease [Kunitz, 1940], and thymonucleodepolymerase [Fischer, Böttger & Lehmann-Echternacht, 1941; Greenstein & Jenrette, 1941], as well as nucleotidases, phosphatases, etc.

Table 2. *Influence of incubation with cockerel plasma on nucleic acids*

0·1 ml. 2% nucleic-acid solution (as Na salt) incubated with 0·2 ml. cockerel plasma and 0·3 ml. veronal-acetate buffer pH 7·6 at 37°.

	Hydrolysis (%)			
	Ribonucleic acid		Thymonucleic acid	
Time hr.	With plasma	Without plasma	With plasma	Without plasma
2	12·2	6·8	0	0
6	36·0	15·0	5·3	1·4
24	74·0	20·4	20·4	7·0

A similar fall in N.P.P. occurs during the first 2 days after the cultures are set up, during which period no measurements are normally made (Fig. 2).

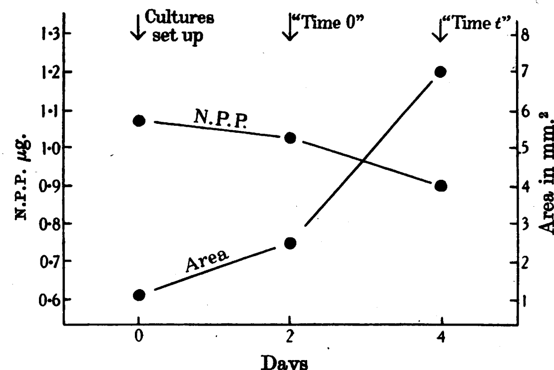

Fig. 2. Decrease in nucleoprotein P (N.P.P.) and increase in area in cultures grown in Tyrode solution alone for 4 days. N.P.P. in μg./roller tube (plasma blanks subtracted). Area in mm.²/individual culture.

Although the amount of nuclear material as measured by the N.P.P. diminishes continuously, the area of the cultures increases and may at the

Table 3. *Effect of various substances on nucleoprotein P* (N.P.P.) *content of tissue cultures*

All cultures grown for 2 days in Tyrode solution alone before introduction of test substance and estimation of initial N.P.P. (X). Final N.P.P. (Y) estimation 2 days later.

	Material under test	Fluid phase in roller tubes	Alteration in N.P.P. µg./tube	$(100Y)/X$
A	Tyrode solution	Tyrode solution alone	−0·15	88
B	Chick embryo extract	Embryo extract	+0·31	120
		Embryo extract after exposure to heat	+0·34	124
C	Crystalline ribonuclease	Tyrode solution alone	−0·14	87
		Crystalline ribonuclease in Tyrode solution (final conc. of enzyme = 9·4 mg. %)	−0·08	91
D	Crystalline ribonuclease	Chick embryo extract	+0·42	137
		Chick embryo extract + crystalline ribonuclease (final conc. = 9·4 mg. %)	+0·35	148
E	Crystalline ribonuclease (Dr Kunitz)	Tyrode solution alone	−0·13	76
		Chick embryo extract	+0·11	114
		Chick embryo extract + crystalline ribonuclease (final conc. = 12·5 mg. %)	+0·09	114
		Tyrode solution + crystalline ribonuclease (conc. as above)	−0·10	82
F	Crystalline ribonuclease (Dr Kunitz)	Tyrode solution alone	−0·07	92
		Chick embryo extract	+0·39	143
		Chick embryo extract + crystalline ribonuclease (final conc. = 50 mg. %)	+0·32	157
G	Crude ribonuclease in pancreatic extracts	Tyrode solution alone	−0·09	92
		Chick embryo extract	+0·45	136
		Chick embryo extract + preparation from pancreatin	+1·03	173
H	Mucinase	Chick embryo extract	+0·44	149
		Chick embryo extract + mucinase (final conc. of mucinase = 168 mg. %)	+0·07	107
J	Mucinase	Tyrode solution alone	−0·08	90
		Chick embryo extract	+0·37	143
		Chick embryo extract + mucinase (final conc. of mucinase = 160 mg. %)	+0·14	116
K	Embryo cartilage extract	Tyrode solution alone	+0·06	106
		Cartilage extract	+0·35	125
L	Anterior pituitary extract	Tyrode solution alone	−0·15	91
		Pituitary extract	−0·10	86

end of 4 days be as much as 6–10 times greater than it was when the cultures were first set up (Fig. 2) owing to the migration of cells from the original explant. The area of cultures from fresh explants is not, however, of great significance [Parker, 1938].

Chick embryo extract as fluid phase

(*a*) *The effect of normal extract.* When the Tyrode solution is replaced by embryo extract, during the second 2 days of the test (i.e. the days of the test proper), very marked growth of the tissue occurs, accompanied by a rise in N.P.P. (Table 3B). The final area, instead of being of the order of 7·0 sq. mm., is of the order of 20 sq. mm./culture.

The rise in N.P.P. is greater as the concentration of embryo extract increases. The effects of different concentrations of embryo extract are shown in Fig. 3. In this case some of the tubes were allowed to run for a further 2 days. During this time no further rise in N.P.P. occurred—in fact a slight fall was observed (Fig. 3). All tests have subsequently been restricted to 2 days after addition of the test substances.

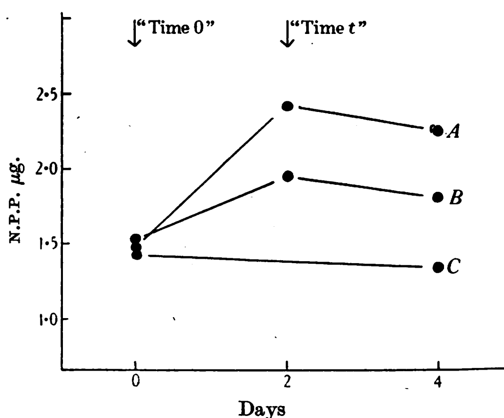

Fig. 3. Influence of different concentrations of embryo extract on the nucleoprotein P (N.P.P.) of tissue cultures. N.P.P. in µg./roller tube (plasma blanks subtracted). All cultures grown for 2 days in Tyrode solution alone before addition of embryo extract. Curve A: fluid phase in roller tube, embryo extract 30 mg. % N (approx.). Curve B: embryo extract 10 mg. % N (approx.). Curve C: fluid phase, Tyrode solution alone. The explants used in this experiment were rather larger than usual.

(*b*) *Effect of heat on embryo extract.* It is usually stated that the active principles of embryo extract are easily destroyed by heat [Carrel, 1913; Lasnitzki, 1937].

We have examined the effect of heating chick embryo extract to 100° for 10 min. To minimize the *p*H changes due to bicarbonate decomposition, the extract, prepared by pulping the embryonic tissue with water instead of Tyrode solution, was divided into two portions, one of which was heated while the other was kept as a control. The salt concentrations (including bicarbonate) in both extracts were then adjusted by adding appropriate amounts of sterile concentrated saline medium. Tissue cultures gained as much nucleoprotein P in this heated material as in the unheated control extract (Table 3B).

(*c*) *The effect of ribonuclease on embryo extract.* In view of Fischer's [1939] suggestion that the growth-promoting power of embryo extract is located in the ribonucleoprotein fraction, we have examined the action of the enzyme ribonuclease on the activity of chick embryo juice. Ribonuclease, which has been prepared in the crystalline state from pancreas by Kunitz [1940], attacks specifically the ribonucleic acids bringing about partial hydrolysis. The enzyme is strikingly thermostable and aqueous solutions can be sterilized by heat.

Chick embryo extract incubated overnight at 37° with crystalline ribonuclease (prepared from cattle pancreas) was tested on the tissue cultures, and showed no diminution in activity as compared with control embryo extract incubated without enzyme (Table 3 C, D). A sample of crystalline ribonuclease kindly supplied by Dr M. Kunitz gave the same result, even when the enzyme concentration in the roller tubes was as high as 50 mg./100 ml. (Table 3 E, F).

Ribonuclease in Tyrode solution alone appeared to have no effect on the living cells (Table 3 C, E). On the other hand, crude preparations of ribonuclease, prepared by extracting commercial pancreatin with 50 % acetone [cf. Dubos & Thompson, 1938], and sterilized by heating to 100° for 10 min., gave a very different response. Cultures grown in a mixture of this medium and embryo extract showed a characteristic dense type of growth with a sharply demarcated margin. Growth appeared to be abundant but cell migration was not so pronounced as in the controls grown in embryo extract alone, which had a considerably larger area. The cultures grown with the addition of the pancreatic extract showed a much greater increase in N.P.P. than did the controls (Table 3G). This increased growth is not caused by the ribonuclease in the pancreatic extracts, but is due to another factor, the nature of which is at present under investigation. It is destroyed by heating for 30 min. at 100° in feebly alkaline solution.

(*d*) *The effect of mucinase on embryo extract.* In view (1) of the large amount of mucin-like substances in embryonic material [cf. Baker & Carrel, 1926], (2) of the good growth-promoting power of embryonic cartilage extracts, and (3) of Fischer's [1940] suggestion that the active principle may contain S, we have tested the effect of a highly purified preparation of the enzyme mucinase [Madinaveitia, 1941] prepared from bull testicle, to see whether hydrolysis of some of the muco- or sulpho-polysaccharides in embryo extract affected its activity. We are grateful to Dr Madinaveitia for a sample of mucinase, which besides acting as a diffusing factor with hyaluronidase activity and with the power to reduce the viscosity of some mucopolysaccharides (of synovial fluid, vitreous humour, and umbilical cord, but not of saliva and gastric mucin), also had a low glucosaminidase activity. Testicular mucinase is stated by Meyer, Chaffee, Hobby & Dawson [1941] to hydrolyse chondroitin sulphuric acid, the mucopolysaccharides of skin, and the polysaccharide (or its H_2SO_4 ester) of the cornea as well as hyaluronic acid. The enzyme was dissolved in Tyrode solution and sterilized by passage through a small Berkefeld candle. A slight loss in N content resulted but the sterile solution still retained powerful enzyme activity as measured by the diffusion test in rabbits with haemoglobin [Madinaveitia, 1938].

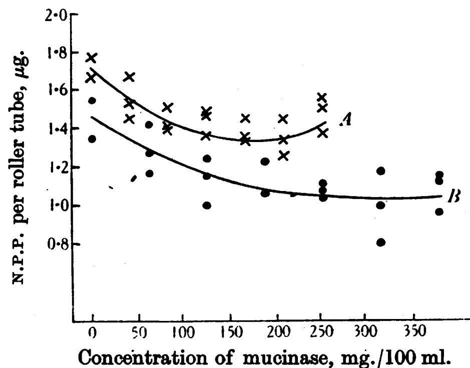

Fig. 4. The effect of increasing concentrations of mucinase on the nucleoprotein P (N.P.P.) of tissue cultures grown in embryo extract. N = 25 mg. % for embryo extract in all cases. Abscissa: N.P.P., μg./roller tube. Ordinate: final concentration of mucinase in roller tube, mg./100 ml. Embryo extract and mucinase incubated for 2½ hr. at 37° before being added to roller tubes. Curve *A* (test 74): cultures grown for 2 days. Curve *B* (test 76): cultures grown for 1 day. Plasma blanks not subtracted. All cultures grown for 2 days in Tyrode solution alone before addition of embryo extract and mucinase.

Embryo extract was mixed with the mucinase solution, and incubated at 37° for 2½ hr. before being added to the roller tubes. Embryo extract incubated at the same concentration without mu-

cinase served as a control. A slight precipitate sometimes appeared in the mucinase mixture.

The cultures grown in embryo extract plus mucinase showed a smaller increase in N.P.P. than did the controls (Table 3 H, J). As the effect seemed to vary considerably with the concentration of mucinase, experiments were carried out in which constant amounts of embryo extract were incubated with increasing concentrations of mucinase. The results are plotted in Fig. 4. It will be seen that the maximum effect was obtained when the mucinase concentrations were 170–250 mg. %. In one test (curve A) higher concentrations were rather less effective.

Extracts of cartilage from mammalian embryos

We have also employed extracts made from mammalian embryos, chiefly sheep embryos of 9–10 weeks, a stage at which growth is very rapid [Gurlt, 1847]. Cattle, pig and human embryos have also been used. As whole extracts prepared by mincing the complete embryos contained an undesirably large amount of blood, we have examined extracts from individual tissues. Embryo cartilage was found to give a very satisfactory extract.

The embryos were dissected and the cartilage of the vertebral column, skull, pelvic and shoulder girdles freed as far as possible from muscle and connective tissue. The cartilage was minced and the mince allowed to stand with an equal volume of water overnight at 0° before being filtered through muslin. The filtrate was centrifuged and treated with ½ vol. of double strength Tyrode solution. To check the salt concentration a chloride estimation was carried out and final small adjustments of salt concentration made as required.

Embryo cartilage extract could be sterilized by filtration through a Berkefeld candle, and kept quite well at 0° or, better, at −30°.

Results obtained with cartilage extract are shown in Table 3K.

The effect of pituitary extract

In view of the well-known action of extracts of the anterior pituitary gland in stimulating the growth of young animals [cf. Young, 1941] we examined the effect of pituitary extracts on the nucleoprotein content of fibroblasts *in vitro*. Saline extracts prepared according to the method described by Marks & Young [1940] were kindly supplied by Dr F. G. Young. The salt concentrations were adjusted to that of Tyrode before the extracts were tested. In no case did the pituitary extract cause any increase in the N.P.P. of the cultures (Table 3L).

This is in agreement with the findings of Trowell & Willmer [1939] who, contrary to Semura [1931], found that pituitary extracts had no growth-promoting power on tissue *in vitro*.

DISCUSSION

It must be emphasized that all the tests have been carried out in fresh explants from the 9-day embryo chick heart and not on pure strains of fibroblasts. The term 'fibroblast' is used here in the usual tissue-culture sense of cells characterized by a network arrangement [cf. Mayer, 1939].

Willmer's [1942] results were obtained mainly with chick periosteal fibroblasts, but in a few experiments with heart fibroblasts he found a fall in N.P.P. in Tyrode solution, while the effect of embryo extract was rather to prevent the fall than to cause a definite increase. In most of our experiments embryo extract produced a definite rise in N.P.P., and this effect was not appreciably diminished with extract which had been heated to 100° for a few minutes, or at 38° overnight. Although Carrel's [1913] original emphasis on the labile nature of the cative principles has been supported by later workers, e.g. Cracium [1931], Hueper, Allen, Russell, Woodward & Platt [1933], and Lasnitzki [1937] who found a decrease in activity on incubation at 70° for 10–30 min., recent workers have reported a greater heat stability, e.g. Paterson [1938]. Tennant, Liebow & Stern [1941] prepared in the ultracentrifuge a growth-promoting fraction from embryonic tissue the activity of which was not diminished by heating at 100° for several minutes. The effect of heated embryo extract on the N.P.P. of pure strains of fibroblasts is, of course, undetermined.

Ribonuclease attacks specifically ribonucleic acids, but its precise mode of action is unknown. Its action may be modified by the presence of protein to which the nucleic acid is bound. For example, Loring [1942] found that ribonuclease was unable to split up the nucleic acid of tobacco mosaic virus—a ribonucleoprotein—so long as the nucleic acid remained attached to the protein, although it could inactivate the virus by forming an enzyme-virus complex containing 14% enzyme. This complex could subsequently be decomposed with liberation of intact and active virus. The claim of Schramm [1941] that intestinal nucleases were able to remove the nucleic acid from the virus leaving the protein intact was not confirmed by Cohen & Stanley [1942]. Although in our experiments ribonuclease even in high concentrations has not influenced the growth of cultures in embryo extract, the participation of ribonucleoproteins as growth-promoting agents cannot be completely excluded since the possibility of the reversible formation of enzyme-ribonucleoprotein complexes remains.

The nature of the factor present in a ribonuclease-containing extract of pancreatin, which causes such a marked increase in N.P.P., is not yet fully elucidated. The effect produced is not merely a quantitative one. Qualitative changes in the type of

growth produced are also found. There is some evidence that the factor may be a P-containing degradation product of protein.

The effect of mucinase is produced only when the enzyme concentration is very high. Both in this case and in the ribonuclease experiments, the enzymes remained active in the roller tubes and one cannot therefore exclude the possibility that, in the case of mucinase, the lower N.P.P. found in presence of the enzyme might be due to a direct action of the enzyme on the cells, rather than an action on the embryo extract. These experiments do, however, suggest that the muco- or sulpho-polysaccharides may be responsible for at least some part of the growth-promoting power of embryo extract.

SUMMARY

1. The effect of various substances on the nucleoprotein phosphorus content (N.P.P.) of fresh explants from the embryo chick heart growing *in vitro* in roller tubes has been examined.

2. Cultures grown in Tyrode solution alone show a fall in N.P.P. The addition of embryo extract to the cultures causes a definite rise in N.P.P., and this rise is unaltered when the embryo extract has been heated to 100° for 5 min.

3. Crystalline ribonuclease has no effect on the growth-promoting properties of embryo extract, but crude ribonuclease preparations from pancreatin contain a factor which causes a marked stimulation of nucleoprotein synthesis in the presence of embryo extract.

4. Cultures grown in embryo extract to which mucinase has been added in high concentration show a smaller rise in N.P.P. than control cultures grown in embryo extract without mucinase.

5. Extracts of mammalian embryos cause an increase in N.P.P. Sheep embryo cartilage extract is particularly effective.

6. Anterior pituitary extracts are without effect on the N.P.P. of the cultures.

We wish to express our gratitude to Mr E. N. Willmer of Cambridge for much valuable advice on tissue culture matters. A grant for scientific assistance from the Medical Research Council and an expenses grant from the Carnegie Trust for the Universities of Scotland to one of us (J. N. D.) are gratefully acknowledged.

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