Table 3. ATP : PHOSPHOINOSITOL PHOSPHOTRANSFERASE ACTIVITY OF ENZYME PREPARATIONS FROM WHEAT ENDOSPERM

Enzyme preparation	Reactants (μmoles per 3 ml. of reaction solution)		Period of reaction at 30° C (min)	ATP determined as glucose-6-phosphate (μmoles per 3 ml. of reaction solution)		Remarks
	ADP	Phytate		I	II	
(*A*) 1 ml.	0	1·0	30	0·44	—	Supernatant fraction from protein body preparation
	0·1	0	30	0·47	—	
	0·1	0·25	30	0·52	—	
	0·1	0·50	30	0·65	—	
	0·1	1·0	30	0·69	—	
(*B*) 0·2 ml.	0	2	30	0·10	0·07	Dialysed protein fraction from whole endosperm; *a* and *b* were additions of 0·4 ml. of enzyme (preparation *B*) which had been pre-treated at 100° C for 30 min with 0·1 N HCl and 0·1 N NaOH, respectively; *c*, phytate added after 60 min incubation
	1·2	0	30	0·25	0·27	
	1·2	2	0	0	0	
	1·2	2	10	0·26	0·22	
	1·2	2	20	0·34	0·27	
	1·2	2	30	0·38	0·40	
	1·2	*a*	30	—	0·46	
	1·2	*b*	30	—	0·41	
	6·0	2	0	—	0	
	6·0	0	60	—	0·68	
	6·0	0	240	—	0·62	
	6·0	2	240	—	1·04	
	6·0	2*c*	120	—	1·14	
(*C*) 0·4 ml.	1·8	0	30	0·68	—	Protein fraction from whole endosperm pre-incubated with ADP and dialysed
	1·8	0·5	30	2·34	—	
	1·8	1·0	30	2·38	—	
	1·8	2·0	30	1·79	—	
	1·8	3·0	30	1·37	—	

The reaction solutions (final volume, 3 ml.) contained the dialysed enzyme preparation (0·2–1·0 ml.), ADP, sodium phytate, magnesium chloride (6 μmoles) and potassium phosphate buffer (100 μmoles) at *p*H 7·4. Formation of ATP was measured enzymatically (refs. 23, 24) after conversion to glucose-6-phosphate with added glucose (200 μmoles) and hexokinase (0·8 mg of Type II, Sigma Chemical Co., United States). Glucose and hexokinase were present during the reaction with the endosperm enzyme in series I, but were omitted in series II. Adenosine monophosphate (AMP) and ADP were also determined enzymatically (ref. 24), and changes in adenine nucleotides were also determined qualitatively after separation by paper electrophoresis (ref. 24).

plant, there is no obvious requirement for active control of the process, and this may be reflected in the apparent stability of the *m*-RNA.

As yet, our investigations have been confined to plant seeds, but it seems likely that structures with similar functions may occur in animal cells, particularly in oocytes of gastropods, amphibia and other animals. These oocytes contain yolk platelets, consisting of protein which is apparently formed in specialized structures[20–22]. These 'Golgi bodies'[21] or 'modified mitochondria'[20,22] may well have similar functions to those of the phytate-containing protoplasts described here.

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INTERFERON RESPONSES OF CHICK EMBRYO FIBROBLASTS TO NUCLEIC ACIDS AND RELATED COMPOUNDS

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IT has been demonstrated that the production of interferon by chick cells exposed to virus is a response to the nucleic acid component[1]. This led to the hypothesis that other nucleic acids might also provide such a stimulus, and we have found that several common nucleic acids will induce monolayer cultures of chick embryo tissue to produce interferon. These observations confirm and extend those recently reported by Rotem, Cox and Isaacs[2], who found that nucleic acids foreign to chick or mouse embryo fibroblasts inhibited virus multiplication when the added RNA was present in cell cultures during infection.

Assays of interferon activity were carried out in tube cultures of chick fibroblasts against vaccinia virus collected from chick embryo tissue cultures. The rubber-stoppered tubes contained 0·9 ml. of Eagle's basal medium with Earle's salts (EBME) maintaining monolayers of 24-h cultures incubated in a stationary position at 37° C. To a series of three tubes was added 0·2 ml. of test material in 2-fold dilution and, after incubation for 24 h, vaccinia virus (10 $TCID_{50}$ per 0·1 ml.) was added. Following three additional days of incubation, monolayers were examined for vaccinia cytopathic effect. The cultures showing no lesions were recorded, the titre of interferon calculated by the Reed and Muench method[3], and expressed as the reciprocal of dilution which would protect 50 per cent of the cultures. When protection was found only in those

tubes receiving 0·2 ml. of undiluted test material, the titre was less than 1, that is, 0·76 or 0·96 with two or all three tubes spared the vaccinial infection.

To prepare interferon, chick embryo fibroblasts were grown in large flat bottles of approximately 600 cm² of surface area. Maintenance fluid was 100 ml. of EBME which for some flasks, as indicated in Table 1, contained 2·0 mg/ml. of yeast nucleic acid or 250 EID_{50}/ml. of type *B*, *GL* strain influenza virus or a combination of both materials. After 24 h at 37° C, an additional volume of 400 ml. of medium 199 was added. In the first experiment, the fluids from all bottles were collected after 13 days, adjusted to *p*H 2·0 with 1 N hydrochloric acid for 1 h at room temperature to destroy virus and then returned to *p*H 7·2 with 1 N sodium hydroxide. From results shown in Table 1, it appeared that an interferon-like substance was produced by cultures treated with commercial grade yeast nucleic acid. A combination of virus and added nucleic acid did not yield more interferon than virus alone. The substances stimulated by virus or non-viral nucleic acid appeared to be identical based on demonstrations of their non-dialysability, resistance to *p*H 2·0 or tryptic digestion, and destruction by heating to 80° C for 30 min, although resistant to lower thermal stress. There was no direct effect of these concentrations of nucleic acids on infectivity of vaccinia when tested in tube cultures.

Table 1. Comparisons of Interferon Titres induced by Yeast Nucleic Acid and Influenza Strain *B/GL* in Monolayers of Chick Embryo Fibroblasts

Stimulant	Bottle No.	Int. titre
Yeast nucleic acid (2·0 mg/ml.)	1	1·9
	2	0·76
	3	1·5
Influenza strain *B/GL*	4	4·8
	5	4·8
	6	3·8
Yeast nucleic acid and *B/GL*	7	6·0
	8	3·8
	9	3·8
None	10	< 0·76
	11	< 0·76
	12	< 0·76

Incubated 13 days before collecting

Several other nucleic acids stimulated anti-viral activity as shown in Table 2. In these experiments the cells were grown in 32-oz. prescription bottles with a 120 cm² surface area. Test materials were sterilized by filtration and used at indicated concentrations in 20 ml. vol. of EBME. Cell sheets were incubated with those fluids for 24 h at 37° C, after which 80 ml. of medium 199 was added, thereby diluting the test material five-fold in 100 ml. of maintenance fluid. It was found that interferon could be stimulated by RNA and DNA or their sodium salts from several sources. With none of these materials was interferon found in the culture fluids until after ten days. If incubation was continued until the majority of cells were degenerate, usually 28–35 days, the interferon titres of aliquots taken periodically reached a maximum at the final collection. Frequently the cultures treated with nucleic acids produced as much or more interferon than did those infected with influenza virus; but the incubation time required was always considerably longer. However, after virus infection, complete degeneration also occurred earlier. In contrast, untreated control cultures maintained for the same period of time without a change of medium contained no interferon activity. On the assumption that the nucleic acids might have induced a latent virus which, in turn, stimulated interferon production, several unsuccessful attempts were made to transfer a cytopathic agent to other cultures or to induce interferon with fluids from the primary culture.

Table 2. Stimulation of Interferon in Chick Embryo Fibroblast Cultures by Nucleic Acids and their Sodium Salts

Materials	Supplier	Conc. (mg/ml.)	Days to collection	Interferon titre
Nucleic acid (yeast)	Calbiochem.	2·5	28	12·0
		1·25	35	15·1
		0·6	35	7·6
DNA (sperm)	Calbiochem.	2·5	28	6·0
		1·25	28	0·6
		0·6	28	1·5
DNA (salmon sperm polymerized)	N.B.C.	0·25	28	1·2
		0·125	28	2·4
		0·06	28	0·76
Sodium DNA (thymus)	Mann	1·0	35	6·0
		0·5	35	1·5
Sodium RNA (yeast)	Mann	2·5	35	6·0
		1·0	35	7·6
Influenza strain *B/GL*		1,000 EID_{50}	13	4·8
Uninoculated control			28	< 0·76

Pursuing these leads further, several purine and pyrimidine derivatives were tested in this system with the results shown in Table 3. Concentrations used were generally lower than those with nucleic acids; nevertheless, several compounds such as deoxyguanalate, deoxycytidylic acid and thymidine were moderately effective in stimulation of the interferon, but none was so effective as yeast nucleic acid. Again this activity appeared only after two weeks of incubation without a change of medium.

Table 3. Stimulation of Interferon in Chick Embryo Fibroblast Cultures by Nucleic Acid Derivatives

Materials	Conc. (mg/ml.)	Days to collection	Interferon titre
Deoxyguanalate	0·5	14	2·4
	0·25	14	1·9
	0·125	28	0·96
	0·06	59	< 0·76
Deoxyguanosine	0·5	25	1·9
	0·25	25	1·2
Deoxycytidylic acid	0·5	25	3·8
	0·25	25	1·9
Deoxycytidine HCl	0·5	25	1·2
	0·25	25	1·9
Thymidine	0·5	25	1·5
	0·25	25	1·9
Thymidylic acid	0·5	25	< 0·76
	0·25	25	0·76
Deoxyadenosine	0·5	46	0·96
	0·25	25	0·96
Deoxyuridine	0·5	18	0·96
	0·25	12	< 0·76
Influenza, strain *B/GL*	3,000 EID_{50}	8	7·6
Uninoculated control		18	< 0·76

It seems likely that production of interferon is a reaction to nucleic acids or their components since addition of several other compounds, as triphosphopyridine, creatine, glutathione and caffeine, to the cultures for long incubation periods failed to yield interferon.

Several facts should be considered in contrasting interferon yields from chick cell monolayers exposed to influenza virus nucleoprotein with that from those cells when treated with nucleic acids or derivatives which were prepared by commercial methods and probably quite degraded. In the case of an efficient viral infection, there is a rapid accumulation within the cell of large polymer viral nucleic acids and precursors. This replication may yield some interferon release in a short time, but the process is often self-limited by destruction of the monolayer. In contrast, when cultures are exposed to a commercially prepared nucleic acid, some of it must first reach an intracellular site.

Initiation of the critical metabolic processes may occur promptly, but a relatively longer time elapses before appearance of interferon in culture fluids. We have found, as did Rotem *et al.*[2], that the cells soon become resistant to infection, which indicates early interferon production. However, the accumulation of significant concentrations of interferon in fluids did not occur until after the cultured cells were in a rather poor state of nutrition, suggesting that interferon may be metabolized by normal, healthy monolayers of cells. It is anticipated that these demonstrations of interferon stimulation by such relatively simple compounds will provide new insight on problems concerned with nucleic acid metabolism.

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