hood (Table 3). As far as the localization in other tissues is concerned, it was found that, besides the intestine, only the stomach, of all the tissues studied, possesses a certain activity (Table 4). In the experiments summarized in Table 5 the intestine of various species of animals, and different intestinal sections from man, were tested. It is evident that, besides the mouse, only the rat possesses, albeit to a lesser extent, the ability to inactivate the virus. No activity was found in horse intestine mucosa. Fig. 1 shows that, at 37° C, the activity increases with incubation time, whereas at 2° C no activity at all could be detected.

From the data presented here, it appears that the principle which inactivates MHV-3 virus is mainly present in the intestinal tract of the adult mouse, and particularly in the first section of the mucosa of the small intestine. Furthermore, it is evident that interaction between the inhibitor and the virus *in vitro* is dependent on temperature.

Work is in progress to determine the chemical nature of the inhibitor and to attain its purification. Preliminary experiments have already revealed the difference between our substance (inactivated at 70° C for 10 min) and the one isolated from the intestine of mice by Mandel and Racker[4] (resistant at 100° C for 10 min).

Table 2. Effect of Various Intestinal Tissue Strata of Adult Mice* on MHV-3 Virus Infectivity

Materials	$-\text{Log } LD_{50}$
Virus	7·4
Virus + intestinal mucosa	2·0
Virus + mucosa-free intestine	4·7
Virus + whole intestine	3·1

* N.M.R.I. strain.

The mucosa was removed with a piece of glass.
For details see text and Table 1.

Table 3. Effect of the Intestine of Mice* of Various Ages on MHV-3 Virus Infectivity

Materials	$-\text{Log } LD_{50}$
Virus	7·3
Virus + intestine, 2-day-old mice	5·9
Virus + intestine, 7-day-old mice	5·0
Virus + intestine, 20-day-old mice	4·6
Virus + intestine, 40-day-old mice	3·5
Virus + intestine, 100-day-old mice	2·4

* N.M.R.I. strain.

For details see text and Table 1.

Table 4. Effect of Various Adult Mouse* Tissues on MHV-3 Virus Infectivity

Exp. No.	Materials	$-\text{Log } LD_{50}$
1	Virus	7·4
	Virus + intestine	3·0
	Virus + stomach	5·0
	Virus + liver	7·4
	Virus + brain	7·3
2	Virus	7·3
	Virus + kidney	7·2
	Virus + spleen	7·0
	Virus + lung	6·9
	Virus + heart	7·4
3	Virus	7·5
	Virus + muscle	7·0
	Virus + pancreas	7·4
	Virus + blood	6·9

* N.M.R.I. strain.

Mice were injected intraperitoneally (0·1 ml.) with 10-fold dilution of each material from 10^{-1} to 10^{-9} (10 mice for each dilution). The experiment reported in Table 4 is one of three which gave similar results.

Table 5. Effect of Intestine of Various Animal Species on MHV-3 Virus Infectivity

Exp. No.	Materials	$-\text{Log } LD_{50}$
1	Virus	7·5
	Virus + mouse intestine*	3·3
	Virus + rat intestine*	4·0
	Virus + guinea-pig intestine*	7·4
	Virus + rabbit intestine*	7·5
	Virus + sheep intestine*	7·3
2	Virus	7·7
	Virus + human duodenum (first portion)	7·7
	Virus + human ileum (last portion)	7·3
	Virus + human vermiform appendix	7·5
	Virus + human colon (first portion)	7·6
	Virus + human rectum (last portion)	7·2
3	Virus	7·4
	Virus + ox intestine*	7·4
	Virus + horse intestine* mucosa	7·5
	Virus + mucosa-free horse intestine*	7·2
	Virus + pig intestine*	6·8
4	Virus	7·5
	Virus + dog intestine*	7·4
	Virus + cat intestine*	7·5
	Virus + chicken intestine*	6·9
	Virus + pigeon intestine*	7·0

* First portion of small intestine.

For details see text and Table 1.

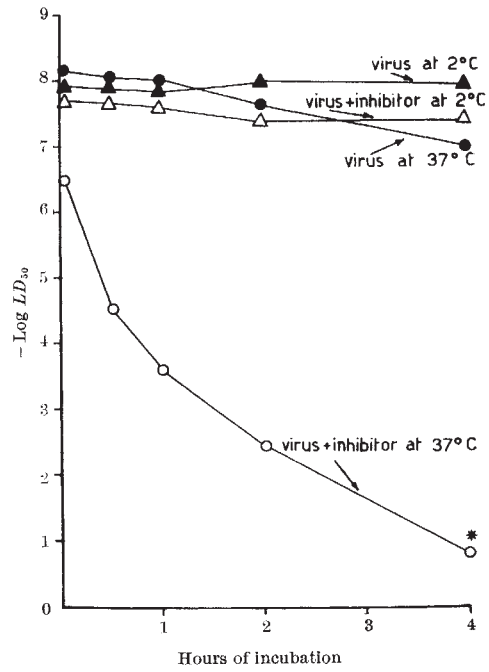

Fig. 1. Interaction between inhibitor and virus at 2° C and at 37° C. Before the addition of the inhibitor to the virus dilutions, the whole system had been equilibrated at 37° C and at 2° C respectively

* $LD_{50} < 10^{-1}$ (at 10^{-1} dilution 2 mice of ten inoculated died).
For details see text and Table 1.

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IMMUNOLOGY

Reaction of Adenine-specific Antibodies with Denatured Deoxyribonucleic Acid

At the present time there is no doubt regarding the role of nucleic acids in carrying genetic information, and their participation in protein synthesis and other biological events is now well known[1]. There is therefore considerable biochemical and genetical importance in the availability of specific antibodies capable of reacting with nucleic acids. The production of such antibodies has been described recently as the result of immunization with DNA that had been complexed with methylated bovine serum albumin[2], by coupling periodate-oxidized nucleotides or nucleosides to proteins[3], or by binding uridine-5′-carboxylic acid to a synthetic polypeptide[4]. Such data promise to make the specificity and sensitivity of immunochemical procedures, which have been of great value in the study of other polymers such as polysaccharides and proteins[5], similarly available for an analysis of nucleic acids.

This communication will report on further observations that support the feasibility of producing antibodies that can react with nucleic acids. It is known that periodate ions are able to attack adjacent hydroxyl groups, cleaving the carbon–carbon bond, yielding aldehydes[6] which can

react with several other groups such as amines[7,8]. In our studies adenine-specific antibodies were obtained by immunizing rabbits with conjugates of periodate-oxidized adenosine coupled to human gamma globulin and albumin, which also had been oxidized by periodate (see Fig. 1).

The conjugates were prepared as follows: 735 mg sodium periodate was dissolved in 20 ml. water; to this solution 184 mg adenosine was added and incubated at 4° C for 12 h. The solution was divided in two 10-ml. portions. To one portion 250 mg of human gamma globulin, dissolved in 5 ml. saline, was added. To the other portion, 250 mg of human albumin dissolved in 5 ml. water was added. Both solutions were incubated at 4° C with stirring for 24 h. During this time a precipitate formed in both solutions. The suspensions were then lyophilized, resuspended and dialysed, and the final volume was adjusted to 10 ml. Since the conjugates were

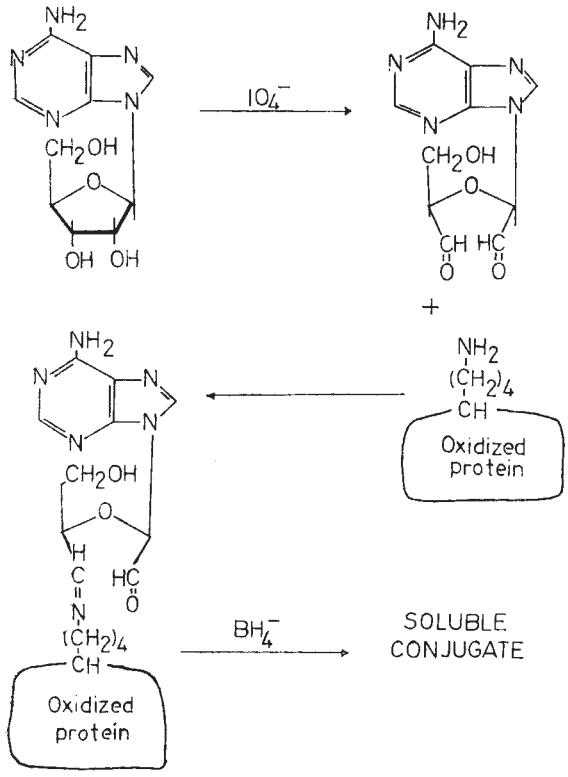

Fig. 1. Periodate oxidation of adenosine, and conjugation

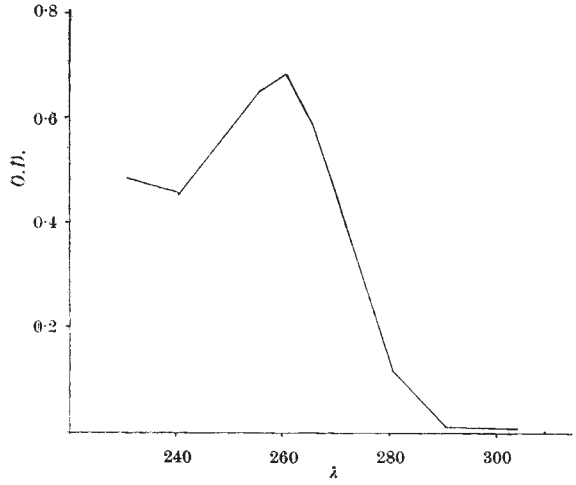

Fig. 2. Periodate oxidized adenosine

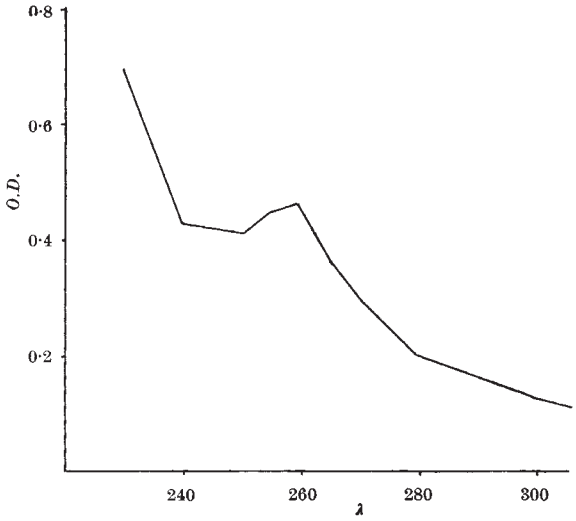

Fig. 3. Conjugate γ-globulin-adenosine (IO_4^-, BH_4^-)

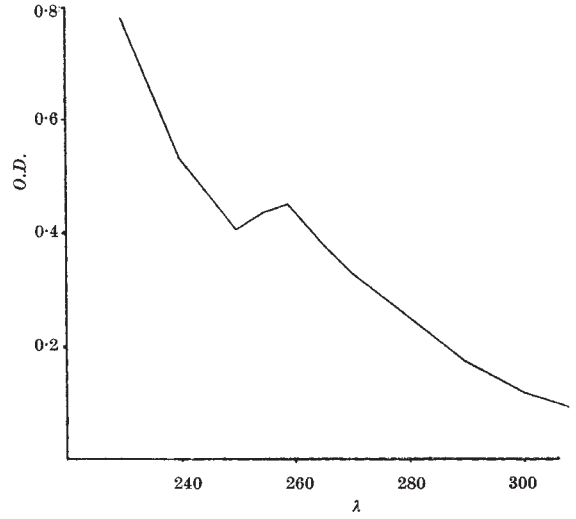

Fig. 4. Conjugate albumin-adenosine (IO_4^-, BH_4^-)

insoluble, they could be washed with water or saline and, when desired, rendered soluble by the addition of sodium borohydride (amyl alcohol was added to avoid foaming). Soluble preparations were used for precipitation reaction, insoluble preparations were employed in immunization. When conjugates were made soluble the excess of borohydride was eliminated by dialysis.

Figs. 2, 3 and 4 show the ultra-violet absorption spectra of periodate-oxidized adenosine and of the solubilized conjugates. According to the absorption spectra of conjugates and oxidized adenosine, the approximate ratio of adenosine to protein was 20–25:1 for gamma globulin and 10–12:1 for albumin.

For immunization, 0·5 ml. of the suspended insoluble conjugates was mixed with 0·5 ml. of adjuvant (Arlacel-Drakeol 1:9+2 mg of *Mycobacterium bovis* per ml.) and injected into the foot pads of rabbits. Two rabbits were used for each conjugate. The animals received five additional intradermal injections at weekly intervals and were bled six days after the last injection.

The sera from the four immunized rabbits precipitated both types (albumin and globulin) of soluble conjugates, but did not react with the heterologous oxidized protein free of coupled adenosine. The four antisera precipitated heat-denatured calf thymus and salmon sperm DNA as shown in Table 1. As shown in Table 2, the amount of antibody that is precipitated will vary with the source of

Table 1. Precipitin Reaction involving Rabbit Anti-adenine Sera* and Deoxyribonucleic Acid Globulin (IO_4^-, BH_4^-), Albumin (IO_4^-), or Conjugates thereof with Adenosine

Rabbits	Immunized with:	Reaction with: GLOB-ADEN (IO_4^-, BH_4^-)	ALB-ADEN (IO_4^-, BH_4^-)	Calf thymus DNA	GLOB (IO_4^-, BH_4^-)†	ALB (IO_4^-)
Rabbit 1	GLOB-ADEN (IO_4^-)	+	+	+	+	(−)
Rabbit 2	GLOB-ADEN (IO_4^-)	+	+	+	+	(−)
Rabbit 3	ALB-ADEN (IO_4^-)	+	+	+	(−)	+
Rabbit 4	ALB-ADEN (IO_4^-)	+	+	+	(−)	+

* Produced after immunization of rabbits with conjugates of periodate-oxidized adenosine and either albumin (ALB) or globulin (GLOB).
† Periodate-oxidation of gamma globulin yields an insoluble product, but it could be solubilized by addition of sodium borohydride.

Table 2. Quantitative Precipitin Reaction with Anti-adenine Serum from Rabbit 1 injected with a GLOB-ADEN (IO_4^-) Conjugate

Source of DNA	Amount added (μg)	Antibody precipitated (as N, ml. 0–2° C) (μg)
Calf thymus (Nutritional Biochemical Corporation)	20	10*
	50	14
	150	28
	300	27
Calf thymus (Sigma)	20	28
Salmon sperm (California Biochemical Research)	20	45

* After addition of 10 mg of adenosine only 3 μg antibody N/ml. is precipitated.

DNA and this may be due to differences in the degree of polymerization of different preparations or, possibly, to differences in the content and location of adenosine in the various heat-denatured DNA preparations.

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BIOLOGY

Experimentation with Plants at Sub-atmospheric Oxygen-levels : Effects of Oxygen Pressure and Salts on Germination of Winter Rye

In a series of recent investigations, it has been shown that plants can be grown in atmospheres with reduced oxygen pressures or in air at reduced total pressure. Germination and seedling growth are in some cases accelerated at sub-atmospheric oxygen levels, and are commonly comparable with these processes in air.

In spite of their essentially normal growth and gross morphology, a number of pronounced changes in biochemical composition[2,3] and metabolism[4,5] have been demonstrated. Some experiments have assessed relationships between pO_2 and specific physiological processes such as abscission[6] or other environmental factors such as heat or cold.

One of the many factors influencing seed germination and seedling growth is salinity. The present paper reports on experiments relating pO_2 and calcium-ion to the effect of saline water on winter rye.

Commercial winter rye seed (*Secale cereale*) (1962 crop) were germinated routinely on filter paper in sterile Petri dishes, containing 25–30 seeds and 10 ml. distilled water. Seeds were incubated at 25° ± 1° C in constant light (cool white fluorescent, 100-ft. candles). Gas mixtures consisted initially of oxygen plus nitrogen.

Rye is a facultative anaerobe, and a highly competent microaerobe, which germinates somewhat more slowly at pO_2 = 15 mm than at higher levels when in distilled water (Table 1). In sodium chloride at 0·25 M, germination at all oxygen levels except 150 mm (air) was reduced to one-half or less relative to distilled water. In 0·75 M, sodium chloride inhibition of germination was complete at pO_2 = 15 mm, and higher oxygen pressures permitted only a few seeds to germinate poorly. At 0·1 M, sodium chloride does not inhibit germination. When $Ca(NO_3)_2$ at 0·02 M is added to this level of sodium chloride, germination at pO_2 = 15 mm is unaffected. In contrast, at all higher oxygen levels in 0·1 M sodium chloride, the presence of calcium nitrate results in significantly elevated germination. When salinity is raised to 0·25 M, $Ca(NO_3)_2$ has a beneficial effect at all oxygen levels, but is relatively more stimulatory at pO_2 50–100 mm.

The most striking response, however, was obtained at 0·75 M sodium chloride, where germination was completely arrested at pO_2 = 15 mm, but proceeds to a slight degree at higher oxygen pressures. At this salinity the calcium effect is seen to be quite large, but quantitatively dependent on the pO_2.

The response of primary root growth (Table 2) post-germination follows the general pattern seen in germination but differs in some significant details.

(a) An optimum pO_2 of 50 mm in distilled water is indicated, but disappears in sodium chloride solutions.

(b) Marked salt inhibition appears between 0·025 and 0·10 M sodium chloride, which is less than required for inhibition of germination.

Again, increasing pO_2 and $Ca(NO_3)_2$ enhances root elongation, and the calcium-effect is aerobic in character.

Table 1. Effect of Oxygen Pressure and Calcium-ion on the Germination of Winter Rye in Salt Solutions

NaCl (M)	pO_2 (mm Hg) 150 − Ca	150 + Ca†	100 − Ca	100 + Ca	50 − Ca	50 + Ca	15 − Ca	15 + Ca
0	83*	83	79	79	60	60	58	58
0·025	83	91	70	76	56	56	56	52
0·100	73	86	71	83	59	78	50	50
0·250	62	83	22	80	23	46	29	40
0·750	(10)‡	88	(7)	59	(7)	18	0	0

* Mean per cent germination after 3 days at 25° C based on replicates totalling 150 seeds. $L.S.D._{5\%}$ = 9%.
† As $Ca(NO_3)_2$, supplied at one-fifth corresponding molarity of NaCl.
‡ (10) denotes emergence = 0·1 cm.

Table 2. Effect of Oxygen Pressure and Calcium-ion on the Root-length of Winter Rye in Salt Solutions

NaCl (M)	pO_2 (mm Hg) 150 − Ca	150 + Ca†	100 − Ca	100 + Ca	50 − Ca	50 + Ca	15 − Ca	15 + Ca
0	2·8*	2·8	2·9	2·9	3·6	3·6	1·3	1·3
0·025	2·4	1·8	2·8	1·7	2·5	1·8	0·8	1·1
0·100	0·8	2·3	0·7	2·7	0·9	2·4	0·5	1·2
0·250	0·3	2·9	0·4	3·0	0·5	2·5	0·4	1·4
0·750	0·1	0·3	0·1	0·4	0·1	0·4	0·0	0·0

* Mean length (cm) of roots after 3 days at 25° C, based on aggregate populations of 35–50 seedlings; $L.S.D._{5\%}$ = 0·7 cm.
† As $Ca(NO_3)_2$, supplied at one-fifth corresponding molarity of NaCl.

Table 3. Relative Effects of Calcium and Nitrate-ions on Root Growth in Salt Solutions at Various Oxygen Pressures

	pO_2 (mm Hg) 150	50	15
Water	2·8*	3·6	1·3
0·1 M NaCl	1·0	0·8	0·3
+ 0·02 M $Ca(NO_3)_2$	3·0	2·8	1·1
+ 0·02 M $CaCl_2$	3·7	1·0	0·4
+ 0·02 M KNO_3	1·1	1·4	0·3

* Mean length (cm) of roots after 3 days at 25° C based on 35–50 seedlings. $L.S.D._{5\%}$ = 0·7 cm.

Table 4. Germination of Rye submerged in Aerated Solutions

Solution	Ionic strength μ	Germination after 10 days (%)
H_2O	—	89
0·7 M sucrose	—	100
0·5 M NaCl	0·50	15
0·5 M KCl	0·50	21
0·18 M $MgCl_2$	0·54	4
0·18 M $CaCl_2$	0·54	87
0·4 M $CaCl_2$	1·2	28