Brit. J. Pharmacol. (1952), 7, 277.

CHEMOTHERAPEUTIC INVESTIGATIONS WITH ROUS SARCOMA VIRUS

BY

LÖIS DICKINSON AND MILDRED J. THOMPSON

From Boots Pure Drug Co., Ltd., Research Department, Bacteriology Division, Nottingham

(Received December 20, 1951)

As part of a programme of investigations into the chemotherapy of virus infections, Rous sarcoma I virus has been used as one of our test viruses. This paper reports the techniques employed and the results for various substances including some known to be effective in the treatment of tumours, e.g. the nitrogen mustards, triethylene melamine, and colchicine. References to these cytotoxic agents appear in recent articles by Loveless and Revell (1949) and Rose, Hendry, and Walpole (1950), and will not be given here. Other compounds were tested because of their action on bacteriophage, e.g. R.D. 347 = 4 : 4′-bis-(2-dihydroglyoxalinyl) stilbene dihydrochloride monohydrate, and R.D. 1367 = 2 : 7-bis-(2-dihydroglyoxalinyl)-9-phenyl-phenanthridine trihydrochloride dihydrate (Dickinson and Codd, 1952), and malabar kino (Chantrill, Coulthard, Dickinson, Inkley, Morris, and Pyle, 1952). Rous sarcoma virus had been grown in fertile eggs by Murphy and Rous (1912) and Keogh (1938), and in day-old chicks by Duran-Reynals (1940); for reasons of convenience in housing animals and in the amount of drug required for treatment attention was therefore directed to the use of these hosts for testing purposes.

Experimental Methods

Strains.—The Rous sarcoma I virus was originally obtained as a freeze-dried tumour specimen from Dr. F. R. Selbie. It was passaged three times intramuscularly in fowls and once on the chorioallantoic membrane of fertile eggs. Egg membrane material was ground in broth and, after centrifugation, the supernatant fluid was filtered through a gradocol membrane of pore size 1.23 μ. This filtrate was the starting point for the work reported here.

Preparation of inocula and maintenance of stocks.—Egg membrane material was passaged 10 times on the chorioallantoic membrane of fertile eggs, 10-day embryos being used and incubation being maintained for a further 7–9 days after inoculation of the virus. Neither whole nor ground membranes maintained their titre for more than one month at $-20°$ when kept in 5 per cent glucose broth and stocks were therefore kept at $-70°$ or freeze-dried. It was later found that the livers of chicks dying from a generalized infection after the intraperitoneal inoculation of day-old chicks were much more satisfactory, for stock preparations, than either membranes or fowl tumours. Such livers were always bacteriologically sterile, were very easy to grind into uniform suspensions, and gave rise to high titre material which maintained its titre indefinitely at $-70°$ or when freeze-dried and for at least one month at $-20°$. For stocks, one liver was

ground with sand in 10 ml. lemco broth and the suspension centrifuged for 15 min. at 2,000 r.p.m. The supernatant fluid gave positive lesions in eggs at a 10^{-3} dilution and the same material, when freeze-dried, was infective at a 10^{-2} dilution. It retained its activity after gradocol filtration (1.14 μ) and was neutralized by fowl antiserum to the original Rous sarcoma.

Tests in ovo.—The technique used is described in detail by Inkley (in press). Fertile eggs from our own stock of Brown Leghorn hens were used. Fourfold dilutions of the compound under test were inoculated into the yolk sacs of groups of 10-day embryos, together with 200 u. each of penicillin and streptomycin. About 3 hr. later the virus suspension was inoculated on to the chorioallantoic membrane. Alternative drug routes, e.g. the allantoic sac and the chorioallantoic membrane, were also employed.

After a further incubation period of 7–9 days, the membranes were removed and examined for the presence of lesions. Even with the minimum of trauma and the use of a technique that gave no "non-specific" lesions with other viruses, control infected membranes did not always respond uniformly. Keogh (1938) found similar variation in individual egg response. It was therefore sometimes difficult to assess visually whether membranes with few large sarcomata were more or less heavily infected than membranes with numerous small lesions. When quantitative assessment was required, all membranes under examination were fixed in formal saline, washed in water, pressed between filter papers to remove excess moisture, and then dried in a hot-air oven at 60° for 24 hr. The dried membranes were then weighed individually and the average weight of each group was compared with the controls.

Tests in young chicks

Strains of chicks.—Brown Leghorns or Light/Heavy crosses from a reliable source were used throughout these experiments. For economic reasons light breed cockerels were generally used, but hens were occasionally substituted with similar results.

Housing and diet.—The chicks were received up to 24 hr. after hatching and put into closed metal brooders, heated by paraffin burners. Throughout the experiment the birds were kept on wire and a standard proprietary national baby chick mixture was fed *ad libitum.*

Testing of drugs.—The age of the chicks at the start of the test varied from 1–6 days as convenient. It was found preferable to use not less than 3-day-old chicks, as they had had time to adjust themselves to new conditions and normal feeding at this age. Each chick was weighed and identified by a wing band. The Rous inoculum, usually frozen or freeze-dried liver suspension, was given either subcutaneously, intraperitoneally, or intramuscularly. After a delay varying from 4 hr. to 10 days, as required, but 24 hr. in the standard test, the administration of the drugs commenced, either the subcutaneous or intraperitoneal route being used. The dose of drug was adjusted according to the body-weight of the chick (checked daily) and the range of dilution had been previously found by toxicity determinations; it included the maximum tolerated dose permitting increased growth of the chicks. The average weight gain for each complete group was calculated over a period of not less than 14 days of treatment, and expressed as a ratio of treated to control birds. Hence a non-toxic compound gave a ratio of 1.0. Chicks weighed about 40 g. at the start of the experiment, and both infected and uninfected birds increased to about 120 g. in 20 days. Since the chicks were growing rapidly, even the slightest toxic effect was apparent. Deaths in the early stages of the test were very few and all are shown in the tables of results; they included deaths due to the toxicity of the drugs as well as those of occasional weak chicks. In these experiments treatment was given once daily (except Sunday) until the experiment ended. Of the drugs employed in the tests reported here, only the antiserum was rendered sterile by filtration. The others

were considerably diluted in sterile saline and no trouble was caused by bacterial contamination. After preliminary tests the following techniques for the assessment of treatment were established for each method of infection used.

Subcutaneous.—The site chosen for the inoculum (0.25 ml.) was the skin covering the peritoneum. Drug doses were administered at the same site or directly into the peritoneal cavity. Birds were killed 9–11 days after inoculation and the parietal peritoneum was examined for the assessment of lesions; values of 1–3 were given according to the number of lesions.

Intraperitoneal.—By inoculating into the peritoneal cavity (taking care to avoid the yolk sac) a generalized infection was produced. The virus inoculum was 0.5 ml. suspension and drugs were normally given by the same route. The spread of the disease was assessed by deaths, survival times, and the macroscopic appearance of the visceral lesions. Each bird was given a maximum value of 10 made up as follows: abdominal organs 2, parietal peritoneal membrane 2, liver 4, death 2.

Intramuscular.—The pectoral muscle was inoculated with virus suspension on either or both sides of the sternum. Drugs were given by the intraperitoneal route. The spread of the disease was assessed by the growth of the tumour (1–4), spread to viscera 4, deaths 2, giving a total maximum of 10.

RESULTS

Subcutaneous.—When the outer skin, with feather follicles, was removed at 9–11 days, the site of inoculation of the virus was exposed. The parietal peritoneum was covered with small, flat, glistening lesions similar to the discrete ectodermal proliferations produced by Rous sarcoma virus when grown on the chorioallantoic membrane of fertile eggs (Plate 1). If the birds were not killed at this stage the lesions developed into tumours, spreading along the peritoneal wall and becoming gelatinous; eventually the tumours broke through the outer skin. On a few occasions when the birds were left on test the growth invaded the viscera and produced necrotic lesions in the liver and peritoneal cavity; this usually happened when there was daily puncture of the peritoneum, as shown in Table II. Carr (1943) stated that chicks infected in the pectoral muscle grew tumours not only at the site of infection but also at other sites, e.g. leg muscle, where a sterile saline injection was made; this demonstrated the tendency for tumours to grow at the site of injury. Since control birds were not usually injected daily, unlike the drug-treated ones, this fact tends to give greater significance to any prevention of tumour growth by a compound. It was not possible to remove and weigh the tumours, and assessment of results was of necessity based on the visual assessment of the lesions at 9–11 days; values of 1–3 were obtained. On the rare occasions when there were no lesions on control membranes, lesions were observed to lie on the under surface of the membrane, presumably carried through by the puncture of the membrane in the intraperitoneal treatment.

To ascertain the nature of these lesions, a suspension of the Rous virus inoculum (liver material), was mixed with an equal volume of undiluted serum obtained from an adult fowl which had been infected a month previously with Rous virus; injected subcutaneously in chicks this mixture produced no lesions on the parietal peritoneum (Table II). An infected peritoneal membrane, showing typical lesions, was removed aseptically from a chick; it was ground in 10 ml. lemco broth and the suspension filtered through a gradocol membrane of pore size 1.23 μ. The filtrate,

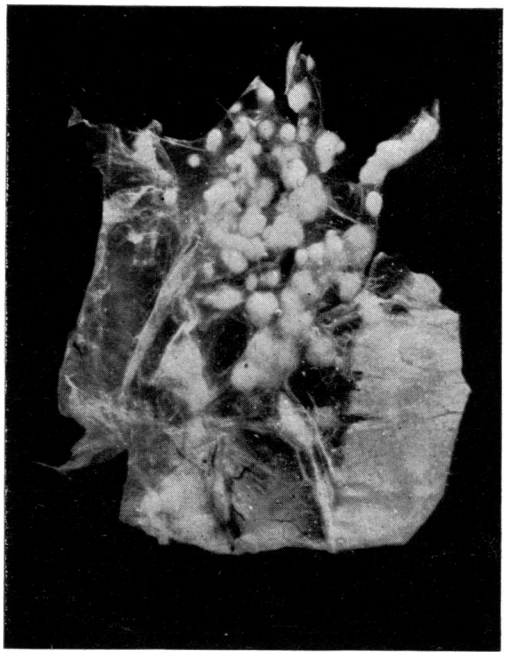

PLATE 1.—Lesions produced by Rous sarcoma virus, 10 days after infection on the parietal peritoneal membrane of chicks. Magnification, × 2.

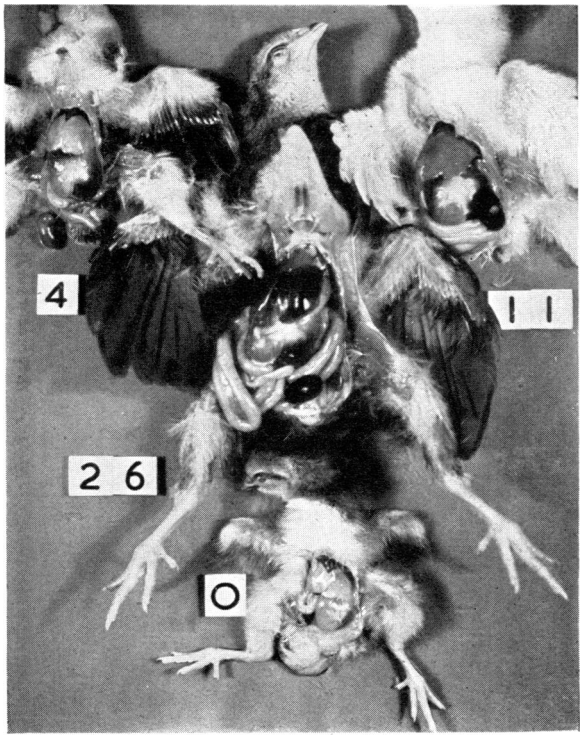

PLATE 2.—Appearance of the yolk sac in normal chicks. 0 = Immediately after hatching. 4 = 4 days old. 11 = 11 days old. 26 = 26 days old. One-third natural size.

which was bacteriologically sterile, was inoculated on to the chorioallantoic membrane of fertile eggs ; the lesions produced were identical macroscopically with those produced by the virus of Rous sarcoma.

Intraperitoneal.—A generalized infection was readily obtained, usually resulting in death within 21 days. Small lesions were noted, especially on the gizzard, early in the experimental period, but it was found preferable to allow the experiment to continue for three weeks, by which time most of the control infected birds were dead. The spread of the disease in the survivors was easily assessed by visual inspection of the viscera ; typical results for control birds are given in Table I. When inoculating the virus intraperitoneally, it was important to avoid the yolk sac, which occupies a large part of the abdomen in day-old chicks ; if the yolk sac were penetrated, there was no spread of infection. For this reason, apart from those reasons already mentioned, it was preferable to use chicks aged not less than about three days. If the yolk sac were pierced deaths occasionally occurred, owing to peritonitis. Another abnormality was the retention of the yolk sac ; such chicks did not gain weight normally. Plate 2 shows stages in the absorption of the yolk sac by normal uninfected chicks.

On necropsy, tumours were regularly found adhering to the parietal peritoneum, particularly at the site of inoculation. They also occurred within the peritoneal cavity, mainly on the intestinal mesentery and on the gizzard. In heavily infected controls the viscera fused into a single unit of tumour tissue, usually accompanied by excessive amounts of clear, gelatinous peritoneal fluid (at this stage death had resulted). The discrete liver lesions were 2–5 mm. in diameter, those on the surface projecting slightly ; in some birds the entire liver was necrotic and was assessed as 4, while in others only one lobe was affected. The evidence for the viral nature of the liver lesions, i.e. the effect of antiserum and filtration, has been given earlier in the paper. The lesions do not appear to be the same as those described by Duran-Reynals (1942), but we have also observed the haemorrhagic lesions in the liver reported by this author.

Intramuscular.—The tumours resulting from intramuscular injection were not easy to remove or measure for assessment. Spread into the lower peritoneal cavity occurred only occasionally, but the liver was often involved. Because of these disadvantages this route of infection was discarded after the early experiments.

Drug action on Rous sarcoma infections in eggs and chicks

Antiserum.—Antiserum to Rous sarcoma virus suppressed the development of lesions on the chorioallantoic membrane when given one hour before or immediately after the virus. It had no effect when given by the yolk sac, allantoic sac, or when given one hour after the virus on the membrane ; by this time the virus had presumably been absorbed by the cells. Similarly, antiserum suppressed the lesions on the parietal peritoneal membrane when given together with the virus. The intraperitoneal results, although not conclusive, followed the same trend. Antiserum had no effect when given daily, commencing 24 hr. after infection.

Triethylene melamine.—This compound was inactive *in ovo*, at the maximum dose tolerated by the embryo (0.01 mg.), when given via the yolk sac or via the chorioallantoic membrane. The chick results, which are summarized in Tables I,

TABLE I

DRUG ACTION ON ROUS SARCOMA INFECTION IN DAY-OLD CHICKS

Intraperitoneal route of infection, followed by intraperitoneal treatment

Dose of drugs	Delay between treatment and infection	Average gain in weight. Treated/control†	No. of chicks	Non-specific deaths or toxic deaths	Individual assessments of lesions. Italics denote deaths	Average assessment of lesions	Value of "p"
Triethylene melamine							
0.3 μg./g. for 17 days	4 hr.	0.87_{14}	6	1	*4*, 2, 1, 3, 1, 1	2.0	<0.001
Controls	—		7	2	4, *8*, *8*, *9*, *8*, *8*, 5	7.14	
0.3 μg./g. for 16 days	24 hr.	1.0_{17}	7	1	1, 1, 2, 0, 1, 1	1.0	<0.001
Controls	—	—	10	0	*8*, 1, 0, 4, *7*, 2, 1, *5*, *9*, 3	4.0	
0.3 μg./g. for 20 days	24 hr.	0.83_{14}	11	3	1, 0, 1, 2, 1, 1, 2, 0	1.0	<0.001
Controls	—		9	1	*8*, *9*, 3, *8*, 5, *9*, 2, 2	5.75	
0.3 μg./g. for 14 days	5 days	—	8	1	2, *8*, 2, *7*, 2, 0, 7	4.0	<0.001
0.3 μg./g. for 9 days	10 days	—	10	1	3, 7, *6*, 2, 6, 1, 4, 3, 8	4.4	<0.001
Controls	—	—	7	0	2, *8*, *9*, *9*, *9*, *10*, *10*	8.14	
Colchicine							
0.6 μg./g. for 3 days	24 hr.	—	12	5*	Test discontinued		
0.3 μg./g. for 20 days	24 hr.	—	8	3*	2, 3, 0, 1, *9*	3.0	<0.001
0.15 μg./g. for 20 days	24 hr.	1.0_{6}	6	0	0, 4, 2, 1, 3, *3*	2.2	<0.001
Controls	—	—	5	0	*8*, *8*, *9*, *7*, *9*	8.2	
Nitrogen mustard (bis-compound)							
0.15 μg./g. for 14 days	24 hr.	0.85_{14}	11	1*	2, *8*, *8*, *9*, 0, *8*, *9*, 0, *7*, *10*	6.1	0.05–0.02
Controls (saline for 14 days)	24 hr. 24 hr.		11	1	*8*, *9*, *9*, *7*, 6, 0, *9*, *10*, *8*, 5	7.1	
Nitrogen mustard (tris-compound)							
0.15 μg./g. for 14 days	24 hr.	0.716_{14}	11	2*	2, 2, *7*, *7*, *5*, *9*, 2, 6, 1	4.5	<0.001
Controls as for the bis-compound						7.1	
Malabar kino concentrate							
30 μg./g. for 16 days	24 hr.	0.94_{17}	10	0	*4*, 6, 4, 2, *5*, 3, 4, 2, 5, 3, *6*	3.95	
Controls as for triethylene melamine (2nd test)						4.0	
Rous antiserum							
1 dose of 0.5 ml. of 1/10 serum ..	0	—	6	0	*8*, 4, 0, *10*, *7*, 0	4.83	0.05–0.02
0.5 ml./chick/day for 9 days	24 hr.	—	6	0	*8*, 6, *8*, *4*, *10*, *7*	7.2	
0.5 ml./chick/day for 19 days	24 hr.	—	6	0	*10*, *8*, *9*, *9*, *7*, 3	7.7	
Controls	—	—	7	1	*10*, *7*, 0, *9*, *10*, *7*	7.2	

* Death due to toxicity of compound.

† Suffix to ratio refers to the period in days over which the average weight gain was calculated.

TABLE II

DRUG ACTION ON ROUS SARCOMA IN CHICKS (SUBCUTANEOUS ROUTE OF INFECTION)

Treatment commenced 24 hr. after infection, except in the case of the antiserum, which was given with the virus

Drug	Route of drug	No. of chicks	Non-specific deaths	Birds examined 9–11 days after infection for lesions on parietal peritoneum		Birds examined 20 days after infection		
				Individual assessments of lesions	Average assessment	Individual assessments of generalized infection	Average	Value of "p"
Triethylene melamine								
0.3 μg./g. . .	S.C.	12	0	1, 1, 0, 0, 0, 0, 0, 0, 0, 1, 0, 0	0.25			
	I.P.	13	0	2, 3, 0, 0, 1, H	1.2	2, 4, 2, 5, *9*, 4, 4	4.3	0.3
Colchicine								
0.15 μg./g.	S.C.	12	1*	0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0	0.0			
	I.P.	13	2	0, 0, 0, 0, 1	0.2	0, 5, 0, 1, 1, 0	1.2	<0.001
Saline controls								
	S.C.	12	1	3, 3, 2, 1, 3, 1, 3, 3, 1, 3, 1	2.18			
	I.P.	13	2	2, 2, 1, 0, 2	1.4	2, 2, *9*, 4, *10*, 3	5.0	
Nitrogen mustard (bis)								
0.15 μg./g.	S.C.	11	0	1, 1, 0, 1, 1, 1, 0, 1, 2, 2, 0	0.9			
	I.P.	11	0	3, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3	3.0			
Nitrogen mustard (tris)								
0.15 μg./g.	S.C.	11	1	2, 2, 0, 1, 1, 1, 0, 2, 2, 0, 0	1.1			
	I.P.	11	0	3, 3, 3, 1, 3, 1, 3, 3, 3, 3, 3	2.6			
Rous antiserum mixed with virus								
	S.C.	5	0	0, 0, 0, 0, 0	0.0			
Saline controls								
	S.C.	11	0	3, 3, 3, 3, 3, 3, 3, 3, 3, 3, 3	3.0			
Malabar kino concentrate								
30 μg./g. . .	S.C.	12	0	1, 1, 1, 1, 1, 1, 1, 3, 1, 1, 1, 1	1.2			
	I.P.	12	2	3, 3, H*, 3, 3,	3.0	*10*, *10*, 5, *10*, 6	8.2	
Controls as for colchicine and triethylene melamine								
	S.C.				2.18	—		
	I.P.				1.4	—	5.0	
R.D. 347								
3 μg./g. . .	S.C.	5	0	2, 1, 2, 3, 2	2.0			
	I.P.	5	0	2, 2, 2, 3, 3	2.4			
R.D. 1367								
3 μg./g. . .	S.C.	5	0	3, 3, 2, 3, 2	2.6			
	I.P.	5	1	1, 2, 2, 3, 2	2.0			
Controls . .	—	10	0	0, 3, 1, 0, 3, 2, 2, 3, 3, 2	1.9			

* Toxic deaths. H = haemorrhagic membrane, not assessed.

II, and III, indicate a considerable suppressive effect, at a non-toxic dose of 0.3 μg./g., on the progress of the disease, even when treatment was delayed for several days after infection. The effect was marked when both drug and virus were given by the same route, i.e. both subcutaneously. When both were given intraperitoneally there was a significant effect, but the treated animals were still heavily infected. In the range of dilutions tested, 3.0 μg./g. was toxic, causing 7/12 deaths within 10 days, 0.6 μg./g. was as active as 0.3 μg./g. and also non-toxic, but 0.15 μg./g. was much less active. When the infection was subcutaneous or intramuscular and the treatment intraperitoneal the action was much less significant.

TABLE III

INTRAMUSCULAR INFECTION FOLLOWED BY INTRAPERITONEAL TREATMENT

Dose of drug	Delay between infection and treatment	Average gain in weight	No. of chicks	Non-specific deaths	Individual assessment of lesions	Average assessment of lesions	Value of "p"
Triethylene melamine							
0.3 μg./g. for 20 days ..	24 hr.	—	11	1	1, *10*, *10*, *7*, 3, *6*, *10*, *2*, *7*, *10*	6.6	<0.001
Controls ..	—	—	11	1	*10*, *10*, *9*, *7*, *8*, *10*, *10*, *10*, *10*, *5*	8.9	

Colchicine.—Colchicine was very toxic to the chick embryo and was inactive at the maximum, tolerated dose (0.0025 mg.), when given by the yolk sac.

Colchicine was the most active compound we tested in chicks. At a dose well tolerated by the chicks and allowing a good gain in weight there was complete suppression of the lesions on the parietal membrane whether the drug was given by the same route as the virus or by a different one (Table II). The results for intraperitoneal infection and treatment were very satisfactory (Table I).

Nitrogen mustards (*bis- and tris-chloroethylamines*).—The bis-compound was inactive *in ovo*, when given by the yolk sac or via the chorioallantoic membrane (0.01 mg.). The tris-compound was not tested.

Both the bis- and tris-compounds, given subcutaneously, had a slight action on the lesions on the parietal peritoneum ; they were inactive when given intraperitoneally. When both infection and treatment was by the intraperitoneal route the tris-compound was significantly active but the bis-compound was much less so. It was noted that, although the chicks gained weight, some liver damage and an occasional death occurred.

Malabar kino was inactive *in ovo* (2 mg.) and inactive in chicks infected intraperitoneally. By the subcutaneous route, the dose employed caused considerable tissue damage, the tannins in the extract being precipitated.

R.D. 347 was inactive *in ovo* (1.57 mg.) and inactive in the subcutaneous chick test at 3 μg./g.; 6 μg./g. gave rise to local tissue damage and results are not reported.

R.D. 1367 was inactive *in ovo* (1.33 mg.) and inactive in the subcutaneous chick test at 3 μg./g.; 6 μg./g. gave similar toxic symptoms to R.D. 347.

Discussion

In this work we have used Rous sarcoma as a virus typifying the tumour-producing group of viruses. It should be stressed that it is not known whether the continued presence of virus, either free or bound, is necessary for the neoplastic nature of the cells or whether the initial infection of the first cell acts as a "trigger mechanism." Consequently one must keep two chemotherapeutic aspects in mind: (*a*) the antiviral action, and (*b*) the anti-tumour action. Rous sarcoma is not commonly used as a test tumour in cancer chemotherapy. The problem was approached by us from the virus aspect, but it was obviously of interest to test known anti-tumour compounds. Fertile eggs are widely used in the chemotherapy of virus infections, but it was felt that the egg test might not respond to cytotoxic agents. Furthermore, the response of the chorioallantoic membrane to Rous sarcoma virus is not as uniform as to other viruses, so that only highly active compounds would be detected by this test. The chick test was therefore developed, and during investigations into the most suitable route of infection it was noticed that the lesions on the parietal peritoneum appeared at about the same time, i.e. 9–11 days, as on the chorioallantoic membrane. The suspicion that these were viral lesions was confirmed by the action of antiserum and the production of typical Rous sarcoma lesions when a gradocol filtrate of the ground parietal peritoneal membrane was inoculated on to the chorioallantoic membrane of fertile eggs.

The intraperitoneal route of infection gave rise to a generalized infection, large amounts of virus being recovered from the liver. The intramuscular infection gave a typical fast-growing tumour which extended to the viscera if allowed to progress beyond a certain time. In adult fowls this dissemination does not often occur and it is possible that antibodies limit the spread. Antibodies were not detected in chicks bled at 20 days, when tested on the chorioallantoic membrane by a neutralization test.

Apart from antiserum, which must be given before the virus has been adsorbed on the cells, no compounds were found active in eggs at a dose tolerated by the embryo for the 7–9 days' duration of the test. However, triethylene melamine, colchicine, and to a slight extent the nitrogen mustards had some action on the lesions of the parietal peritoneum of chicks, where a larger amount of drug could be tolerated than in the chick embryo. R.D. 347 and R.D. 1367 were quite inactive and malabar kino caused such damage to the parietal peritoneal membrane that the reduced assessment was not considered significant. It was inactive in the intraperitoneal test. Only colchicine suppressed the lesions completely and it was also the only compound to suppress them when given by the intraperitoneal route. It is possible that triethylene melamine and the nitrogen mustards did not reach the required concentration in the parietal peritoneal membrane when given by the intraperitoneal route. The results for the intramuscular infection with intraperitoneal treatment confirmed this view.

Colchicine, at 0.15 μg./g., was effective when the intraperitoneal route of infection was used, but it should be stressed that 0.3 μg./g. was toxic. Triethylene melamine was significantly active in repeated tests, even when given several days after infection; for this drug the therapeutic index was between 4 and 10. The nitrogen mustards were much less active and also toxic. As expected, antiserum was inactive

when given 24 hr. after infection. Even when given immediately after infection there was little effect; this, however, was expected, since the antiserum was considerably diluted and dispersed in the peritoneal cavity as opposed to the subcutaneous route.

Malabar kino was included in this test merely as an example of a toxic drug. In general one would not consider, as a possible anti-virus drug, any compound markedly toxic to the host cells. Unfortunately, only cytotoxic agents have shown activity in our tests. There are as yet no known anti-virus drugs, except the newer antibiotics, which are only active against the larger viruses; this has limited the work considerably. R.D. 347 and R.D. 1367, although active against bacterial viruses, are very toxic to animals; they were included because the test on the parietal peritoneum seemed to offer the best chance of any activity being detected. The activity of these two drugs is not inhibited by protein, unlike that of malabar kino, where proteins precipitate the active principle, presumably a tannin.

The fact that antiserum can prevent the development of infection, if given before or immediately after infection, indicates that the chick test would be expected to pick out a compound acting against the virus as well as those acting against the tumour cell metabolism. With the cytotoxic agents the chick test revealed activity which was not detected by the egg test. Moreover, the subcutaneous route of infection enables results to be assessed almost as quickly as in the egg. The technique is simple and is proving satisfactory as a screening test.

No detailed pathological or histological studies were made in this work, in view of the approach to the problem as a chemotherapeutic study of a *virus* infection, using a typical tumour-producing virus.

Conclusions

1. Techniques for the chemotherapeutic studies of Rous sarcoma virus are described, using fertile eggs and young chicks. In the latter, viral lesions can be assessed on the parietal peritoneal membrane at 10 days.

2. Only antiserum, given before or immediately after infection, was active in the egg test.

3. Cytotoxic agents, particularly triethylene melamine and colchicine, were active in the chick tests; the nitrogen mustards were less active.

The authors wish to acknowledge the interest of Sir Jack Drummond, F.R.S., and Mr. C. E. Coulthard in this work. They thank Mr. J. Bennett for a constant supply of eggs and chicks and Mr. G. Whiting for the photographs.

References

Carr, J. G. (1943). *Brit. J. exp. Path.*, **24**, 127.
Chantrill, B. H., Coulthard, C. E., Dickinson, L., Inkley, G. W., Morris, W., and Pyle, A. H. J. (1952). *J. gen. Microbiol.*, **6**, 74.
Dickinson, L., and Codd, S. (1952). *J. gen. Microbiol.*, **6**, 1.
Duran-Reynals, F. (1940). *Yale J. Biol. Med.*, **13**, 77.
Duran-Reynals, F. (1942). *Cancer Res.*, **2**, 343.
Inkley, G. W. In press.
Keogh, E. V. (1938). *Brit. J. exp. Path.*, **19**, 1.
Loveless, A., and Revell, S. (1949). *Nature*, **164**, 938.
Murphy, J. B., and Rous, P. (1912). *J. exp. Med.*, **15**, 119.
Rose, F. L., Hendry, J. A., and Walpole, A. L. (1950). *Nature*, **165**, 993.