the observations on strains 119 and MP. The figures for differential neutralization derived from these results are

Between strains 39 and 119 (Vallée O and A types)	4.6
Between strains 39 and 149 (Vallée O and Waldmann C types)	5.0
Between strains 119 and 149 (Vallée A and Waldmann C types)	3.3
Between strains 119 and MP	1.9

Thus only in the case of strains 119 and M.1 does the magnitude of a strain difference nearly equal a type difference.

Discussion. The antibody produced in response to infection appears to differ qualitatively from strain to strain of those examined. The importance of these differences in immunization may depend on a number of factors. The antibody response of an animal to an injected vaccine may not reproduce qualitatively the response to an infection even if the preparation of vaccine from a particular strain has preserved the antigenic peculiarities of that strain. Again the quantitative factors influencing immunization may render strain specificity less important than it would at first appear. Further comment must be deferred until observations have been accumulated on these aspects. Strains shown by cross-immunity tests in vaccination experiments to be more closely related than those mentioned in this paper require to be examined, and work on these lines is continuing.

Summary. (1) Differences between strains of the virus of foot-and-mouth disease of one type have been demonstrated by cross-neutralization tests in cattle. (2) The magnitude of such differences in these experiments is not so great as that between immunological types.

16622

Strains of the Virus of Foot-and-Mouth Disease Recovered from Outbreaks in Mexico. Vaccination Experiments.

W. M. HENDERSON, IAN A. GALLOWAY, AND J. B. BROOKSBY.
(Introduced by R. E. Shope.)
From the Research Institute (Foot-and-Mouth Disease Research Committee), Pirbright, Surrey, England.

Experiments on the immunization of cattle against infection with strains of virus from Mexico were made to determine whether vaccines prepared from the highly immunogenic Pirbright stock cattle strain 119 of the Vallée A type would protect them against infection with these strains which are of the same type. The results of these early experiments showed a difference in the antigenic behavior of strain 119 and the Mexican strain MP. This observation led to a study of these and other Mexican strains by methods described elsewhere.[1,2] Observations were made also on the immunizing value of vaccines prepared from the Mexican strains themselves. The problem became complicated later by failure of these Mexican strains to infect susceptible cattle, in control groups included in contact infection tests, with the regularity associated with highly invasive strains.

The pattern of the experiments was the same in each case. Vaccines were prepared from different strains, groups of cattle were injected with these vaccines and 2 to 3 weeks later, the protection established was tested with the same strain or another strain of the same immunological type.

Methods. Particulars of the strains of virus

[1] Brooksby, J. B., Galloway, Ian A., and Henderson, W. M., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 70.

[2] Brooksby, J. B., Galloway, Ian A., and Henderson, W. M., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 74.

MP, M.1 and 119 used in these experiments have already been given.[1,3]

Preparation of Vaccines. Vaccines prepared by the inactivation of infective blood were chosen for these experiments. The use of this type of vaccine as a prophylactic in foot-and-mouth disease was reported first by Graub, Zschokke and Saxer[4] and the general principles of its preparation have since been followed at this Institute in developing inactivation tests, vaccine potency tests and for studying the difference in antigenicity of strains of virus and in other immunological problems.

Four or five litres of blood were collected from the jugular vein of each of a group of 2, 3 or 4 cattle that had been inoculated on the tongue with freshly passaged virus material.

The cattle were bled between the 24th and 48th hour after inoculation at a time when secondary lesions were developing. The blood was defibrinated, the samples pooled and a 1% solution of Du Pont "extra pure" crystal violet added to give a final concentration of 0.05%. The inactivation treatment consisted of 6 or 8 days incubation at 37°C. The titre of the pooled blood was determined by our routine method using cattle.[5] Vaccines prepared from each new strain studied were tested for inactivation of the virus after the requisite period of incubation at 37°C. The criterion for inactivation or non-infectiveness was the recording of consistently negative results in 80 to 120 observations made on the sites of multiple tongue inoculations of a group of 8 to 12 cattle.

Vaccination of cattle. Groups of Devon steers, 1½ to 2 years old were vaccinated by subcutaneous injection into the dewlap in the region of the brisket. The vaccines were usually stored at 4°C for a short time after completion of incubation at 37°C. In no case was this period longer than 23 days. In other experiments the potency of this type of vaccine has been proved to remain unaltered for at least 4 months at either 4°C or frozen at −20°C.

Exposure of Vaccinated and Control Cattle to Infection by Contact. The cattle were housed in loose boxes 12 feet square and in contact exposure tests one vaccinated or unvaccinated control animal was left in a box with an infected animal for at least 14 days. This virus donor was a susceptible animal which was inoculated in the tongue with virus at the time the 2 animals were placed together.

Tongue Inoculation of Vaccinated and Control Cattle. The inoculum in each case was a 0.55μ gradocol (Elford) membrane filtrate of the supernatant fluid of a centrifuged suspension of freshly collected vesicle epithelium. The filtrate was titrated in cattle and a dilution made to provide an inoculum of the desired strength. Each animal was inoculated intradermally on the tongue at 10 separate sites using 0.1 cc at each site. The result of a titration is based on similar inoculations of 0.1 cc amounts. Thus if the 50% positive end-point of the inoculum was 10^{-4} then each of the 10 sites on the vaccinated or control animals' tongue would receive approximately 10,000 50% end-point infective doses *i.e.* 10,000 I.D. 50.

Examination of Animals. The mouth and feet of the cattle were examined daily, at first, and later on every second or third day for 3 weeks after contact exposure and for 2 weeks after inoculation.

Results. Experiment A. Contact exposure test with strains 119 and MP vaccines and viruses. Strain 119 Vaccine No. 93—Titre of blood $10^{-3.6}$.

Strain MP Vaccine No. 94—Titre of blood $10^{-3.2}$.

The results of previous experiments (as yet unpublished) had shown that from 6 of 8 to 8 of 8 cattle which had received a 30 cc dose of a strain 119 vaccine resisted infection by contact with virus donors inoculated respectively with Vallée A type strains 119, A. Cor.

[3] Brooksby, J. B., Henderson, W. M., and Galloway, Ian A., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 64.

[4] Gräub, E., Zschokke, W., and Saxer, E., *Schweiz. Arch. Tierheilk.*, 1939, **81**, 436.

[5] Henderson, W. M., 1945, Thesis, University of Edinburgh, (in press as an Agricultural Research Council Report).

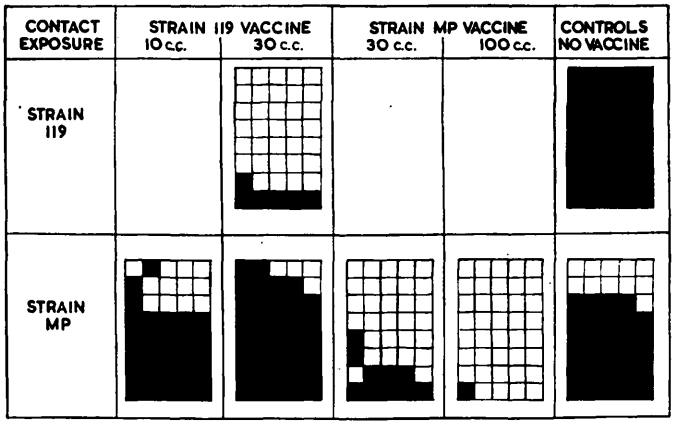

FIG. 1.

Experiment A. Contact exposure test with strains 119 and MP vaccines and viruses. In this and subsequent figures each large rectangle depicts a group of cattle with a horizontal line of five squares for each animal. The first square represents the tongue and/or lips and the next four squares the four feet. A lesion at any particular site is denoted by blackening the appropriate square.

and AX. In the present experiment, 2 groups of cattle injected respectively with 10 cc and 30 cc doses of vaccine No. 93 (strain 119), were exposed to infection by contact with virus donors inoculated with strain MP virus. As a control of the potency of the vaccine another group injected with 30 cc were exposed in a similar way to infection with strain 119. Vaccine No. 94 (MP) was tested by exposing two groups of cattle injected respectively with 100 cc and 30 cc doses to infection with strain MP virus. The results of the experiment represented diagrammatically in Fig. 1 show that although vaccine 93 (strain 119) gave the expected degree of protection against the 119 strain, an equivalent amount of vaccine did not protect any of 8 cattle against infection with strain MP. A clinically recognizable disease did not develop in all of the control group of susceptible cattle exposed to infection by contact under similar conditions and this point is discussed further when the results of the next experiment are dealt with. The results show also that the protection conferred on cattle by the strain MP vaccine against infection with strain MP was not of such a high order as that given by a strain 119 vaccine against infection with strain 119.

Experiment B. Contact exposure test with strains 119 and MP vaccines and viruses.

Strain 119 vaccine No. 96—Titre of blood $10^{-3.7}$.

Strain MP vaccine No. 95—Titre of blood $10^{-1.5}$.

It has already been mentioned[6] that the titre of the blood of our cattle infected with Mexican strains tended to be somewhat low. As the titration of infective material only assesses the amount of active virus, a low titre might not necessarily indicate the true antigenic level. It was for this reason that strain MP vaccine No. 95 was used in this experiment even although the titre of the blood was much lower than our usual standard for vaccine preparation.

The experiment was similar to experiment A. A dose of 100 cc of the strain 119 vaccine was injected into one group of cattle to determine whether increased dosage would compensate for the apparent antigenic dissimilarity between strains 119 and MP in exposure to infection against strain MP and another group received 30 cc doses as a link with the previous experiment.

[6] Henderson, W. M., Galloway, Ian A., and Brooksby, J. B., PROC. SOC. EXP. BIOL. AND MED., 1948, **69**, 66.

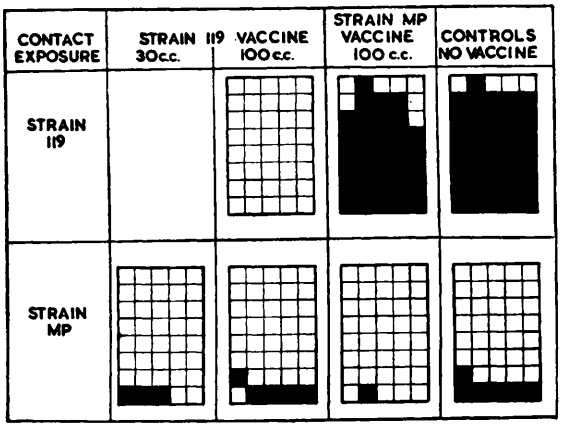

FIG. 2.
Experiment B. Contact exposure test with strains 119 and MP vaccines and viruses.

The strain MP vaccine was tested against infection with strain 119, as well as against infection with strain MP.

The results are shown in Fig. 2. The failure of 6 of the 8 cattle in the unvaccinated MP control group to react to infection renders negative results valueless in interpreting that part of the experiment involving exposure to infection with strain MP. The facts that 6 out of 8 controls as against the 2 out of 8 of the last experiment failed to react to exposure to infection and that 3 of the 6 non-reactors when subsequently tested for susceptibility by tongue inoculation reacted like fresh susceptible cattle suggests a change in the behavior of strain MP from experiment to experiment. This is perhaps associated with a variation in the invasiveness of the strain. The altered conditions in the present experiment no doubt account for the fact that only 1 of 8 as against 8 out of 8 cattle in *experiment A*, injected with a 30 cc dose of strain 119 vaccine reacted when exposed to infection with strain MP.

The results of the exposure of vaccinated cattle to infection with strain 119 showed that the protective value of the strain 119 vaccine was up to expectation. The complete failure of the strain MP vaccine in a dose of 100 cc to protect cattle against infection with strain 119 is unfortunately impossible to interpret as the other half of the experiment provided no satisfactory estimate of its potency against infection with the strain homologous virus MP. More extended observations are necessary to determine whether this lack of protection of cattle by the strain MP vaccine, under discussion, against infection with strain heterologous virus 119 could be attributed to the low titre of the blood used in preparing the vaccine or was a real antigenic dissimilarity.

Two facts had arisen during the course of this work. Firstly that clinical reactors to the Mexican strains of virus did not always yield blood of adequate titre for vaccine production. Secondly that these strains were unsuitable for use in contact infection experiments owing to their inability to produce visible signs of foot-and-mouth disease in all the cattle of an unvaccinated control group.

Accordingly, in the next experiment a vaccine was prepared by adding a filtrate to infective blood to ensure having starting material of good titre. Also it was decided that the method of testing for immunity would be altered to the intradermal inoculation of the tongue with a filtrate of known virus potency in the hope that this would be followed by complete reactions in the animals of the control groups. Sufficient data have not been accumulated yet to show how the results of the tongue inoculation of vaccinated cattle can be correlated with those of infection by contact exposure tests. A preliminary experiment, Fig. 3, with a highly in-

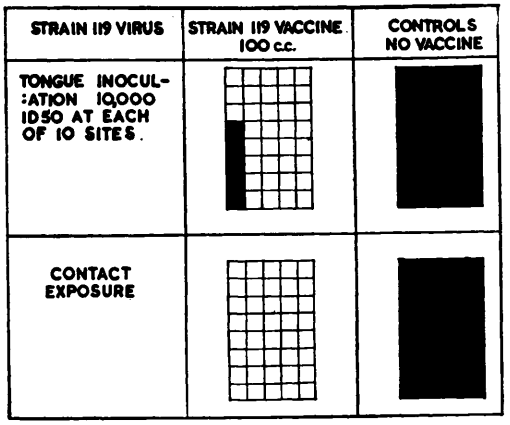

FIG. 3.
Comparison of tongue inoculation with contact exposure of test animals in a vaccination experiment. Strain 119 vaccine and virus.

vasive strain, 119, showed that when the dose of vaccine injected is large, protection against development of secondary lesions in inoculated cattle corresponds to complete protection against contact infection.

Experiment C. Tongue inoculation and contact exposure test using strain M.1 vaccines and virus.

Strain M.1 vaccine No. 102—Titre of blood $10^{-2.8}$.

Strain M.1 vaccine No. 103—Titre of blood $10^{-2.8}$ with the addition of a filtrate of an epithelial suspension of tongue vesicular lesions to give a final titre of $10^{-3.7}$.

Two different inocula were used, one having a titre of $10^{-4.7}$ and the other a 1:1000 dilution of the first, having a titre of $10^{-1.7}$. The doses of the vaccines injected were 100 cc. The results are shown in Fig. 4. While the complete absence of secondary lesions in all the vaccinated groups demonstrates the high degree of protection that can be conferred on cattle by this type of vaccine prepared from this strain of virus, it did not permit the relative merits of the two vaccines to be assessed. Nor did it allow the demonstration of the advantage of giving 2 successive smaller doses of vaccine instead of one large one. This improved effect has been demonstrated in work (unpublished) with other strains of virus when the successive doses of vaccine were less than in the present instance.

It will be noted also that there was little difference in the effects produced respectively by the small or the large amounts of virus in the two inocula. It will be appreciated that once a primary lesion is produced the

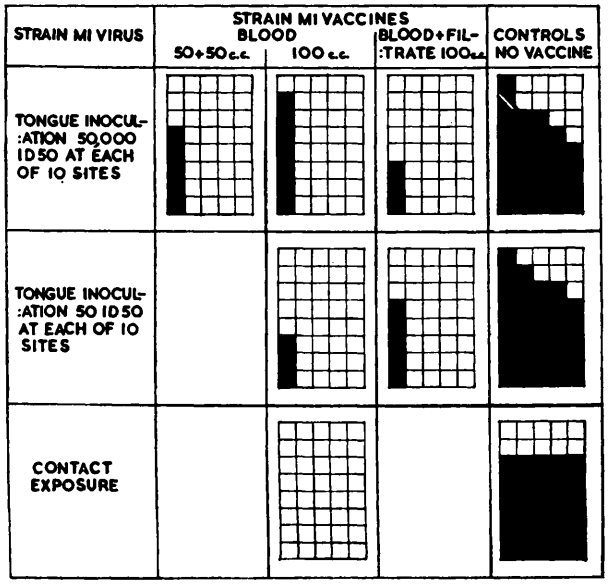

FIG. 4.
Experiment C. Tongue inoculation and contact exposure test with strain M.1 vaccines and virus. A white diagonal stripe denotes a secondary lesion on the lip following a primary lesion on the tongue.

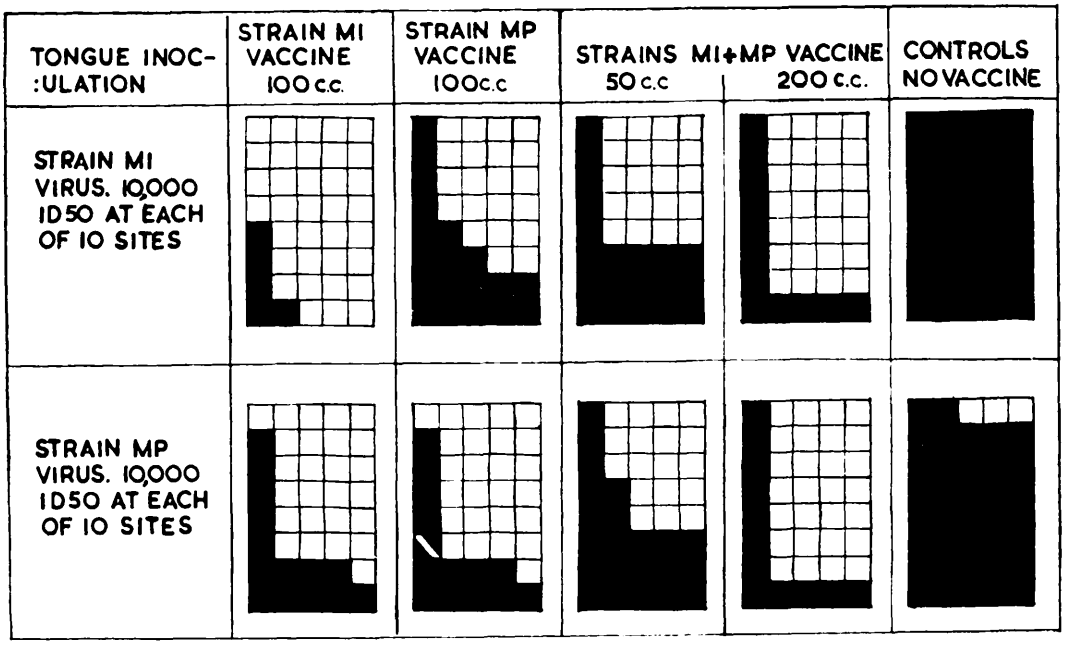

FIG. 5.

Experiment D. Tongue inoculation test with strains M.1 and MP vaccines, a "bivalent" vaccine incorporating strains M.1 and MP, and strains M.1 and MP viruses. A white diagonal stripe denotes a secondary lesion on the lip following a primary lesion on the tongue.

quantity of virus originally inoculated bears no relation to the severity of the test for the animals have then to contend with the greatly increased amount of virus released from the focus of multiplication. The non-appearance of a primary lesion in a vaccinated animal following the tongue inoculation of virus is undoubtedly of significance. Nevertheless the production of a primary lesion must constitute a severe test of the animal's immunity.

A contact exposure test was carried out at the same time and the cattle of the control groups of the tongue inoculation test were used as virus donors. Eight cattle injected with 100 cc of vaccine No. 102 were exposed to virus infection by contact and there was a group of 8 unvaccinated control cattle.

Although here again the incomplete reaction of the cattle of the control group complicates the picture to some extent, the result of the comparative test confirms that shown in Fig. 3 in the experiment in which strain 119 was used.

Experiment D. Tongue inoculation test using strain M.1 and MP vaccines, a "bivalent" vaccine incorporating strains M.1 and MP, and strain M.1 and MP viruses.

Strain M.1 vaccine No. 105—Titre of blood 10^{-3}.

Strain MP vaccine No. 106—Titre of blood $10^{-2.6}$.

Strain M.1 + MP vaccine No. 107—a mixture of equal quantities of vaccines Nos. 105 and 106 made at the completion of the incubation of each component.

This experiment was planned as the first of a series to determine the significance in the immunization picture of the differences recorded in the complement fixation and serum neutralization tests between strains M.1 and MP. The titres of the respective inocula of the 2 strains were approximately the same *viz.* 10^{-4}. Primary lesions were produced in the majority of the animals and the estimate of the efficacy of the vaccines was based largely on the non-development of secondary lesions. Although each vaccine gave better protection against the strain of virus used in its preparation than against the other, Fig. 5, the differences in the results between the

groups were not of greater magnitude than to indicate more than a trend in the direction of some antigenic dissimilarity between strains M.1 and MP such as that strongly suggested by the results of their examination in complement fixation and serum neutralization tests. This dissimilarity may have been masked by the large dose of vaccine administered. Further observations are necessary in experiments in which a series of varying doses of vaccine will be used before full significance can be attributed to the results of the different methods of examination of strains of virus.

The results show that the lesser protection conferred by the strain MP vaccine is consistent with the lower titre of the blood used in its preparation. The "bivalent" vaccine incorporating both strains induced a comparable resistance against infection with either strain when the dose was adequate.

Summary and Conclusions. The low invasiveness of the Mexican strains of virus used in the vaccination experiments described with the resultant incomplete reaction of the control groups of cattle made the interpretation of the results difficult. Nevertheless 88 of a total of 96 cattle *i.e.* 92% which had received a dose of 100 cc of blood vaccine (with crystal violet added), in which the method of inactivation of the virus is by incubation at 37°C, showed no lesions in contact tests or no secondary lesions in tongue inoculation tests when the challenge strain or strains of virus (bivalent vaccines) were those used for preparing the vaccine. Even if all tests are included *i.e.* tests in which the doses of vaccine were 100 cc or only 30 cc or 25 cc or the challenge Mexican strain of virus was not the same as that used for vaccine production 110 of 136 *i.e.* 81% showed equivalent protection. This compared with the 12 of 56 *i.e.* 21% of non-vaccinated control cattle which remained free of all lesions in contact tests and secondary lesions in tongue inoculation tests.

As a comparison a series of observations (unpublished) with strain 119 made in a similar way may be cited. In these experiments 72 of a total of 72 cattle *i.e.* 100% injected with 100 cc of a strain 119 vaccine remained free of all lesions in contact tests and secondary lesions in tongue inoculation tests when tested with strain 119. If 68 cattle which received only 30 cc of vaccine are added, 133 of a total of 140 *i.e.* 95% similarly remained free of lesions. These figures can be compared with 2 of a total of 88 *i.e.* 2.3% of animals in the unvaccinated control groups which similarly remained free of lesions.

These figures indicate the effectiveness of the type of vaccine employed in these experiments and give some idea of the good immunogenicity of the Mexican strains of virus.

The difference in antigenic behavior between the Mexican strains M.1 and MP was not as clear cut as those recorded in the complement fixation and serum neutralization tests.

More comparative data are required to interpret the significance of the antigenic dissimilarity between strains indicated by the different methods of examination.

It is noteworthy that although the Mexican strains under review were clearly classifiable in the Vallée A immunological group of foot-and-mouth disease virus, a potent vaccine prepared from the Pirbright stock Vallée A type strain 119 did not protect cattle against infection with strain MP virus under the conditions of the experiment.*

* Since this paper was submitted for publication the question of whether a large dose of a strain 119 vaccine would mask this antigenic dissimilarity has been investigated further. The opportunity was taken also to study the antigenic relationship of strain 119 and strain M.1. In a tongue inoculation experiment three groups of eight cattle vaccinated respectively with 100 cc doses of a strain 119 vaccine were inoculated intradermally in the tongue 2½ weeks later with strain 119, strain MP and strain M.1 viruses respectively. In the group tested with strain 119 (10,000 I.D. 50) six animals showed no reaction and two had primary lesions with no development of secondary lesions. In the group tested with strain MP (2,500 I.D. 50) and strain M.1 (2,500 I.D. 50) all of each group of eight animals developed primary lesions and, in each case, five of the eight developed secondary lesions; 1 foot, 2, 2, 4 and 4 feet respectively in the MP group and 2, 4, 4, 4 and 4 feet in the M.1 group. All the cattle in the unvaccinated control groups showed complete devel-

This result emphasizes the necessity in attempting prophylactic control of Mexican virus infection, of testing vaccines prepared from Vallée A strains other than selected Mexican strains under controlled experimental conditions before using them in the field.

opment of primary and secondary lesions; strain 119, 16 cattle; strain MP, 8 cattle; strain M.1, 8 cattle.

This result shows that a large dose of a strain 119 vaccine is insufficient to mask the antigenic dissimilarity between strain 119 and the Mexican strains MP and M.1 and that the tongue inoculation test as well as the contact exposure test can be used to demonstrate these differences in antigenic behavior.

16623

Effect of BAL on Cobalt-induced Polycythemia in Rats.

LEON O. JACOBSON, EDNA K. MARKS, AND EVELYN GASTON.

From the Argonne National Laboratory and the Department of Medicine, University of Chicago, Chicago, Ill.

Introduction. In preparation for a study of the effect of BAL (British Anti-Lewisite) on the biological effects and the excretion of radioarsenic (As^{76}), an experiment was designed to determine the influence of this chemical agent on the development of cobalt-induced polycythemia in rats. The mechanism of cobalt-induced polycythemia is not understood.

Barron *et al.*[1] demonstrated a decreased respiration (Warburg) of hemopoietic tissue from cobalt-stimulated animals. This was not corroborated by Warren *et al.*[2] Recently Burk *et al.*[3-5] have shown that the toxic action of cobalt is related to its effect on –SH groups. It seemed worthwhile, therefore, to determine whether or not BAL, which is effective in the protection of –SH groups from arsenical poisoning, would prevent cobalt-induced polycythemia in rats.

Material and Methods. Young Sprague-Dawley rats weighing approximately 300 g each were divided into 5 groups and prepared as follows: Group I (5 rats) served as a control; Group II (5 rats) received 0.2 mM/kg of BAL in peanut oil intramuscularly 3 times weekly; Group III (15 rats) received cobaltous chloride subcutaneously 5 times weekly (0.357 mg calculated as Co); Group IV (5 rats) received 0.2 mM/kg of BAL 3 times weekly; and Group V (5 rats) received cobalt as above but the BAL was begun 6 weeks after the cobalt was initiated.

Two control hemoglobin and hematocrit (Kato) determinations were made on all rats prior to starting the BAL or cobalt injections and at biweekly or monthly intervals thereafter.

Results. As is indicated in Fig. 1 and 2 the animals which were given BAL and cobalt from the beginning of the experiment, the animals which were given cobalt from the beginning and BAL 6 weeks later, and the animals which were given only cobalt developed a comparable polycythemia (increase in hemoglobin and hematocrit values) in approximately 8 weeks. The hemoglobin and hematocrit values of control animals and animals that received BAL only remained essentially constant.

Discussion. The dose of BAL used in this

[1] Barron, A. G., and Barron, E. S. G., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 407.

[2] Warren, C. O., Schubmehl, G. S., and Wood, I. R., *Am. J. Physiol.*, 1944, **142**, 173.

[3] Burk, D., Schade, A. L., Hesselbach, M. L., and Fischer, C., *Fed. Proc.*, 1946, **5**, 126.

[4] Burk, D., Hearon, J., Caroline, L., and Schade, A. L., *J. Biol. Chem.*, 1946, **165**, 723.

[5] Burk, D., Hesselbach, M. L., Fischer, C., Hearon, J., and Schade, A., *Cancer Research*, 1946, **6**, 497.