but not especially purified) are undoubtedly minute. While some species of the genus *Candida* are known[8] to produce sufficient riboflavin on controlled low-iron media to colour the medium, we have never succeeded, using the strains of *Candida albicans* employed here, in obtaining more than very small yields of riboflavin with similar, purified, known-iron media.

While it is well known that the presence of external phosphate is not essential for the oxidation and assimilation of glucose by yeast suspensions[9], it is also known that phosphate exchange[4,6] and polymerization (as measured by the increase in basophily[11]) are dependent on the presence of external metabolizable substrate.

In view of the correlation which has been pointed out[3] between the action of 2,4 dinitrophenol on the assimilation of carbon sources by micro-organisms and its action on the uptake or exchange of inorganic phosphate by micro-organisms, it is of interest to compare the action of riboflavin with that of other agents on these two processes. Such a comparison has been made in Table 3 (references noted). It appears there is a decided 1 : 1 correlation between the processes of phosphate uptake and assimilation of carbon by micro-organisms. At the concentrations stated, the agents listed in Table 3 are without effect on the rate of aerobic or anaerobic breakdown of carbon sources. The effect noted[10] for riboflavin on assimilation was observed with the two organisms reported on in this paper. From concomitant investigations[10] on the variation in basophily of yeasts (see Wiame[11]), it is believed that the 1 : 1 correlation between assimilation and phosphate uptake results from the, possibly coupled, simultaneous polymerization by yeasts of inorganic phosphate (to polyphosphates) and of carbon sources (to carbohydrate polymers).

TABLE 3

Substance	Concentration	Assimilation of carbohydrate	Uptake or exchange of inorganic phosphate
2,4 Dinitrophenol	10^{-4} *M*	inhibited[3]	inhibited[3]
Sodium azide	10^{-4} *M*	inhibited[3]	inhibited[3,13]
Gramicidin*	40 μgm./ml.	inhibited[3]	inhibited[3]
Glucose	10^{-3} *M*	a substrate	enhanced[4,6]
Riboflavin	$2{\cdot}5 \times 10^{-6}$ *M*	enhanced[10]	enhanced

* Acting on *Staphylococci*, other agents acting on yeast.

The observation of Malm[12] that phosphate exchange by yeast is sensitive to the *p*H of the medium can be taken as evidence that a surface reaction, easily affected by external *p*H, is involved in phosphate exchange (see Myrbäck and Vasseur[14] for an extended discussion of the considerations underlying this line of reasoning). It is unlikely that phosphate enters the yeast cell in an ionized form, and it seems reasonable that phosphate is brought through the cell barrier by uniting with some 'complexing substance' existing at the cell surface. The compound of this substance with phosphate may then dissociate, or in other manner lose phosphate to the cell (possibly to coenzyme I; see Lindahl *et al.*[5]), and free 'complexing substance' to act again. From our observations we believe that such a role as postulated for the 'complexing substance' may be filled by riboflavin. Combined roles of phosphorus and hydrogen transference for trace-active substances may assume importance in considerations of *in vivo* processes.

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FACTORS INVOLVED IN THE DEACTIVATION OF PENICILLIN SOLUTIONS BY RUBBER TUBING

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COWAN[1] in 1945 demonstrated that aqueous solutions of penicillin could lose up to 50 per cent of their antibiotic activity by simple passage through the rubber tube of a continuous-drip apparatus. These results were confirmed by Huelsbusch, Foter and Gibby[2], who showed that certain samples of both natural and synthetic rubber could inactivate completely the penicillin in solution in twenty-four hours; and also by Hughes[3], who showed that no improvement was obtained by repeated washing of the tubes before use.

At the request of the Medical Research Council through the Ministry of Supply Advisory Service on Rubber, we have been investigating this problem in the hope that we should be able to throw some light on the mechanism of inactivation and on possible methods of improving the quality of rubber supplies. The main technological details will be published elsewhere, and the purpose of this communication is to give a summary of the results relevant to medical practice.

The Effect of Compounding Ingredients and of Vulcanization. Unfortunately, none of the early workers was in a position to supply information on the compounding ingredients other than rubber which were present in the tubes which they tested. These ingredients may well represent quite a large proportion of the total weight, and as some of them are highly active substances it was thought possible that the trouble might be due to one or more of these rather than to the rubber itself. On testing singly a number of such substances, including natural rubber, sulphur, zinc oxide, stearic acid, paraffin wax and a number of accelerators of vulcanization—mercaptobenzthiazole, dibenzthiazyl disulphide, tetramethylthiuram disulphide, zinc diethyldithiocarbamate and diphenylguanidine—no loss in penicillin potency was observed with any of these materials. However, when these ingredients were compounded together in various proportions to give products suitable for rubber tubing and then subjected to vulcanization, the resulting rubber tubes were found to be very active towards penicillin. Thus a rubber tube vulcanized with sulphur and 1·0 per cent of mercaptobenzthiazole reduced the potency of penicillin by 80 per cent in 24 hours at room temperature, and in no case was the loss less than 60 per cent in this period.

Causes of Penicillin Deactivation. It is clear from the above that the active agents in penicillin degradation by rubber must be produced during the complex chemical changes occurring during vulcanization. The major reaction which results in the cross-linking of the rubber molecules with sulphur is unlikely to leave the latter in an active form; but in the process of heating with sulphur, it is not improbable that mercaptan compounds could be produced from any non-rubber components present. This is particularly likely in the case of vulcanization accelerators, many of which decompose during vulcanization with the production of mercaptan compounds. It is known that cysteine is effective in the deactivation of penicillin, and indeed it has been suggested by Cavallito[4] and others that penicillin and other antibiotics inhibit the growth of micro-organisms by reaction with mercaptan compounds within the organism.

In the first instance, therefore, the effect of a reduction in the proportion of accelerators from 1·0 per cent to 0·1 per cent was studied. In each case a substantial corresponding reduction was achieved in the activity of the rubber towards penicillin. Thus in the case of dibenzthiazyl disulphide, the loss of potency of the penicillin was reduced from 80 to 40 per cent, while with a diphenylguanidine-accelerated mix reduction was from 74 to 20 per cent. By the omission of accelerators altogether, a still further reduction was obtained. In some cases the rubber showed no deactivating effect whatever, whereas in others varying effects up to a maximum of 20 per cent were observed.

Confirmation of this finding was obtained by the simple heating of an accelerator—in this case tetramethylthiuram disulphide—to vulcanization temperature for ten minutes. After this treatment the product was found to be highly active towards penicillin, in contrast to its initial inert behaviour. It is therefore clear that accelerators play a major part in the deactivation of penicillin by rubber, and it is clearly desirable that the amounts used should be as small as possible.

The smaller, and variable, activity of vulcanized rubbers containing no accelerator would appear to be due to the reaction of sulphur with impurities rather than with the rubber itself, as otherwise it would be difficult to explain the preparation of some mixes which are entirely inert towards penicillin. Impurities which could react to form mercaptan compounds are often present in small amounts in natural rubber in the form of the so-called 'rubber resins', and some evidence that these may play a part in penicillin deactivation was obtained by testing acetone extracts of vulcanized rubbers prepared without accelerators. Rubber resins and their reaction products with sulphur are largely soluble in this solvent whereas the rubber complex is not, and such extracts were found to be active towards penicillin in those cases in which the vulcanized rubber itself showed activity.

Methods of Minimizing the Degradation of Penicillin. It has already been shown by Yudkin and Pulvertaft[5] that vulcanized rubber is without action upon penicillin in the presence of phosphate buffer, and we have found this to be true even with the most active rubbers which we have been able to prepare. However, where the use of phosphate buffer is impracticable, the potential destructive action of mercaptans will constitute a danger in any form of vulcanized rubber, whether natural or synthetic. In studying possible methods of minimizing this effect, we have obtained some success by the use of a suitable grade of carbon black as an inert filler in the compounding of the rubber tubes. Thus, the incorporation of carbon black in an accelerated mix resulted in the rubber tube showing 27 per cent loss of penicillin potency in 24 hours, whereas an exactly similar rubber tube, containing the same volume proportion of barytes, resulted in a loss of potency of 78 per cent. Comparable results were obtained with a variety of different accelerators. The effect is due presumably to the absorption of active substances and the prevention of their migration to the surface.

The second method examined was the treatment of vulcanized rubber tubes with reagents which are active towards mercaptan groups. This aspect has not yet been studied extensively, but promising results have been obtained by treatment for a few hours with 40 per cent formaldehyde at 38° C., whereby the destructive power for penicillin of a highly active rubber tube was considerably reduced (from 91 to 68 per cent loss of potency in 24 hours). Similar results have been obtained on a variety of different mixes, suggesting that this method of treatment would be worthy of further study, while at the same time lending some support to the view that it is the mercaptan groupings in the vulcanizate which are the active principles concerned in the destruction of penicillin.

Effect of Repeated Sterilization and of Ageing. Small chemical entities such as simple mercaptans are known to migrate to the rubber surface. This suggested a danger that the destructive power of rubber for penicillin would increase with age, and this aspect has also been examined. No alteration in the deactivating power of rubber tubes could be observed by repeated sterilization; but tubes which have been subjected to normal storage for a period of six months were found to show a marked increase in their activity towards penicillin. These tubes were deliberately prepared without the usual antioxidant in order to accelerate any effects due to ageing; but this finding does suggest the desirability of occasional checks upon rubber tubing during its period of use or storage.

Alternative Materials. In view of the obvious difficulties in the preparation of rubber tubes which can be relied upon to be completely without action upon penicillin, we have investigated the effect of a number of alternative materials. Of those which we have studied, polyvinyl chloride, with the plasticizers normally used in its manufacture, and polyethylene, have no action whatever upon penicillin. Polyethylene has the disadvantage that it shows a tendency to kink when used in long lengths, and both materials present difficulties in sterilization as their softening points are below the temperature of the normal steam autoclave. However, polyvinyl chloride can be made in a form resistant to boiling water and is already finding uses in surgery, so that it may prove to be superior to rubber for the purpose of the drip-feed injection of penicillin.

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LONDON'S TRANSPORT SYSTEM

THE fourteenth annual report, recently issued by the London Passenger Transport Board, makes interesting reading. Since the undertaking is now vested in the British Transport Commission, this is the Board's final report, and it has therefore devoted one section to a review of the way in which it has discharged its functions since July 1933.

Here is an example of the operation of a very large centralized concern run on lines similar, from many points of view, to those which must be adopted in the even larger organisation of nationalized industries or utilities. There can be no doubt that in this instance the arrangement has proved in most ways an outstanding success: the Board has, in the main, achieved its object of providing "a passenger transport service, by rail and road, worthy of London as a great metropolitan city". Few Londoners would deny this: it is not so certain that the inhabitants of some other capital cities would be equally enthusiastic about the transport facilities available to them.

The magnitude of the Board's achievement can better be appreciated by reference to some of the figures contained in the report. The area served covers some 2,000 square miles, with a population in 1933 of 9,358,000. One of the first tasks was to rationalize the unrelated and often wasteful competitive services operated by the many transferred undertakings. During the pre-war years the number of vehicles owned rose from 11,753 to 12,819, and the annual mileage run by them increased from 515 to 573 million miles, providing more than nine million passenger journeys a day in 1933 and ten million in 1939—the usual calculation indicates that if the tickets issued daily were placed end to end they would stretch from London to New York. The receipts from fares rose concurrently from about 27 million pounds to nearly 31 million pounds sterling a year. While this expansion was in progress, the many types of buses and coaches were being replaced by standard designs, the maintenance system was overhauled, new equipment was introduced to increase reliability and efficiency and, most important of all, a vast programme of new works was undertaken in conjunction with the 'main line' railway companies

The outbreak of war caused a great setback to this steady growth. As a result of evacuation, the 'blackout' and bombing, the volume of traffic was so reduced that the system ceased to be self-supporting. But after 1941, traffic again increased and reached a peak of 4,259 million journeys in 1946, totalling 11,479 million passenger miles. The number of journeys made per head of population per year is a measure of the 'travel habit'; this figure rose from just over 100 before the War to nearly 130 in 1946, which certainly indicates no reluctance on the part of the public to travel.

Mention may be made of some of the technical innovations originating with the Board. In 1938 re-designed rolling stock was introduced on the 'underground' railways, the electric motors being installed beneath the floors of the carriages. This eliminated the waste of one car in a seven-car train to house the power plant, and thus gave a 14 per cent increase in carrying capacity. The Board was among the first to recognize the advantages of the oil engine as compared with the petrol engine from the point of view both of performance and of economy in operation and maintenance. It accordingly adopted the oil engine from 1934 onwards as the standard power unit for all new buses and coaches. It was also a pioneer in the large-scale use of the pre-selective gear box and fluid flywheel transmission, which not only ease the work of the driver but also materially increase the comfort of passengers by virtue of the smoother running which they give. Before the War, the trams on many routes were replaced by trolleybuses; but since the War the financial advantage of the trolleybus has decreased to such an extent that further conversion of tramways will be to buses, which are more flexible in operation and offer wider opportunities for co-ordination of all services provided by the use of a single type of road vehicle. Another remarkably successful innovation of the Board was the novel constructional technique employed on the eastern extension of the Central Line.

The first regular passenger service in London was established in 1829, and during the succeeding century development proceeded under a multiplicity of private operators. The extraordinary complexity of the resulting system makes it difficult to offer a fair criticism of the Board's efforts, though it would appear that there is room for immediate improvement in certain directions: the rationalization of routes in the central area and, in particular, the links between the 'main line' railway stations require attention. It is, for example, impossible to get from King's Cross to Victoria station without at least one change, apart from the interstation buses which are so infrequent and circuitous in route as to be virtually useless.

The report concludes with a reference to future prospects and deplores the lack of materials and national restrictions which have prevented a more rapid recovery from the effects of war. It has been argued, however, that the present difficulties are attributable, at least in part, to the policy pursued before the War of scrapping vehicles when the body was worn out, instead of retaining the chassis with a new body and an oil engine; this criticism applies particularly to the 'ST' type of motor-bus. But the Board would probably be the first to admit that errors of judgment have occasionally been made and to acknowledge that the traffic system is still capable of much improvement.

Though the layman may occasionally wonder at some of the oddities of the system—what purpose is served, for example, by the posters informing us that London's transport is at our service when no other means of travel is available, and why does the hand-rail always travel slower than the escalator?—the Board is to be congratulated on furnishing an example of the efficiency which should be attained by all public undertakings. R. H. MACMILLAN