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# The effect of Arvin on reticulo-endothelial activity in rabbits

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## Summary

1. Arvin stimulated the reticulo-endothelial uptake of colloidal carbon in rabbits. The onset of effect after intravenous administration was immediate and the minimum stimulant dose by this route was very low—0·05 unit/kg (0·1 μg protein/kg).

2. Carbon opsonized *in vitro* with serum taken 4 h after an intravenous injection of Arvin was cleared more rapidly than carbon treated with control serum. Serum collected 10 min after Arvin did not promote carbon uptake.

3. Reticulo-endothelial block with Thorotrast or excess carbon did not prevent the stimulant effect of Arvin but prevented the stimulant effect of opsonization with 4 h serum.

4. The reticulo-endothelial stimulant effect of Arvin appears to be unrelated to its coagulant action on fibrinogen. The mechanism may involve a direct action on phagocytic cells and an effect on colloidal particles via serum opsonins.

## Introduction

Arvin is a fraction obtained from the venom of the Malayan pit viper, *Agkistrodon rhodostoma,* by chromatographic separation (Esnouf & Tunnah, 1967). It has a specific coagulant action on fibrinogen and when administered in a therapeutic dose to man and experimental animals causes a rapid lowering of plasma fibrinogen and the blood becomes incoagulable. The effects of crude venom and Arvin in man and experimental animals have been described (Reid, Chan & Thean, 1963; Regoeczi, Gergely & McFarlane, 1966; Marshall & Esnouf, 1968; Bell, Pitney, Oakley & Goodwin, 1968; Sharp, Warren, Paxton & Allington, 1968; Ashford, Ross & Southgate, 1968; Reid & Chan, 1968; Regoeczi & Bell, 1969).

During the course of some experiments on the anti-inflammatory effect of Arvin (results to be published) we observed that carbon was cleared more rapidly from the blood of Arvin-treated rabbits than from that of controls. These observations prompted an investigation of the effect of Arvin on reticulo-endothelial activity using the clearance rate of colloidal carbon as the criterion of effect.

The results, reported in this paper, show Arvin to be a potent stimulant of the reticulo-endothelial system (RES) in rabbits.

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**Methods**

Colloidal carbon (Gunther Wagner Batch C11/1431a) was obtained as a suspension containing 160 mg carbon/ml and 4·3% fish gelatin as a suspending agent. Thorotrast, a colloidal suspension containing 24–26% thorium dioxide by volume and 25% dextrin as a stabilizing agent, was used as an RES blocking agent.

Ovomucoid was obtained from Sigma Ltd., bovine serum albumin from B.D.H. Ltd., and fibrinogen and streptokinase from Kabi Ltd.

Arvin was made by the method of Esnouf & Tunnah (1967) and was supplied by G. W. Tunnah of Twyford Laboratories. The dose is expressed in units, each unit containing approximately 2 $\mu$g of protein.

Anti-Arvin serum was supplied as purified goat serum globulin by Dr. Parrish of the Lister Institute. Goats were immunized by intramuscular injections of whole crude *Agkistrodon rhodostoma* venom. Tolerance to the venom was first induced by giving a series of increasing doses in saline. These were followed by treatment with the material in a water-in-oil emulsion and finally by further injections of venom in saline. The serum globulin fraction was purified by pepsin digestion and salt fractionation. Potency was assessed in terms of the capacity of the antiserum to neutralize the coagulant effect of Arvin *in vitro*. Antiserum was incubated with excess antigen (Arvin) for 30 min and the residual Arvin activity was then measured by means of a clot test on human plasma. One unit of antiserum is therefore the amount required to neutralize 1 unit of Arvin *in vitro*.

Male New Zealand White rabbits weighing 1·5 to 2·5 kg were used throughout.

Intravenous injections were made into the marginal ear vein and intramuscular injections were made into the thigh. Blood samples were taken from the marginal ear vein by venepuncture.

*In vivo* phagocytosis was measured by the rate of clearance of intravascular colloidal carbon. Timed, 0·1 ml blood samples were obtained following the intravenous injection of 80 mg carbon/kg into conscious rabbits. The sample was lysed by dilution in 2·5 ml 0·1 M $Na_2CO_3$, and the carbon concentration measured photometrically at 650 nm.

The rate of carbon clearance is an exponential function of the concentration to time (Stuart, Biozzi, Stiffel, Halpern & Mouton, 1960; Biozzi, Benacerraf & Halpern, 1953).

The results are expressed as intravascular clearance half-times, and are calculated from plots of log carbon concentration versus time.

*Opsonization of carbon*

This was carried out by the method of Jenkin & Rowley (1961). Carbon, at a concentration of 32 mg/ml in 2% gelatin, was mixed with an equal volume of serum and incubated at 37° C for 20 min just before injection. Antiserum (1 u./ml) was added to the incubation mixture. The dose was 5 ml/kg given intravenously.

*Preparation of fibrinopeptides*

A mixture containing the fibrinopeptides split off from fibrinogen by Arvin was prepared as follows. Lyophilized fibrinogen containing sodium chloride was

dissolved in 100 ml distilled, deionized water to give 100 ml of a 1% fibrinogen solution in 0·9% saline. To this was added 150 u. of Arvin, and the mixture was left at room temperature for 6 h to coagulate. The clot was then squeezed over a No. 2 sinter and the supernatant collected.

This supernatant, which contains the fibrinopeptide A and derivatives released by Arvin (Ewart, Hatton, Basford & Dodgson, 1969) may also contain unchanged Arvin. Accordingly, an excess of anti-Arvin serum (7 u./ml) was added to the supernatant. Each rabbit then received 5 ml/kg intravenously of this mixture. A control batch was prepared, omitting the fibrinogen, to ensure that all Arvin which may have been present was neutralized by the antiserum.

*Preparation of fibrin degradation product mixture*

Citrated rabbit plasma was obtained by collecting blood from the ear vein into 20% v/v 3·8% trisodium citrate. 50 u. of Arvin were added to 10 ml of plasma. This was incubated at 37° C for 1 h in a test tube containing a glass rod with a roughened end. The rod was rotated for half a minute every 5 min during the first half-hour so that the clot adhered to the roughened end of the rod. At the end of an hour the clot was squeezed gently against the side of the test tube until approximately 6 ml of supernatant fluid was left in the test tube. The rod with the attached clot was then transferred to a test tube containing 10 ml Michaelis's barbital-acetate buffer, pH 7·4, and 5,000 u. streptokinase/ml and was incubated at 37° C for 24 h, by which time all the clot had been lysed. To this mixture was added 10 u./ml of anti-Arvin serum in order to inactivate any unchanged Arvin which may have been present.

A control was prepared by incubating 3 ml of normal rabbit serum containing 5 u. of Arvin/ml with 10 ml of Michaelis buffer containing streptokinase, for 24 h. To this was added 10 u./ml of anti-Arvin serum.

The dose was 5 ml/kg intravenously.

## Results

*RES stimulant effect of Arvin (Table 1)*

A single intravenous or intramuscular dose of Arvin caused a profound increase in the rate of carbon clearance, the effect lasting for 48 to 72 hours. The threshold dose was 0·05 u./kg intravenously and stimulant effects were obtained up to 1 u./kg, this being the highest intravenous dose given. Following 1 u./kg it was not possible to demonstrate a latent period before the onset of stimulation. Tolerance to the stimulant effect of Arvin did not develop following four daily intramuscular or three daily intravenous injections.

*Effect of anti-Arvin serum on stimulant action of Arvin (Table 2)*

When 1 u. of Arvin was treated with 2 u. antiserum, the usual defibrinating effect of this dose of Arvin was prevented: that is, blood samples taken after the test clotted normally, but the RES stimulant effect was still present. A large excess of antivenom (0·1 u. Arvin–2 u. antiserum) neutralized the effect of Arvin on the RES. The Arvin and antiserum were mixed vigorously just before injection.

*Effect of Arvin in RES blocked animals (Table 3)*

RES blockade was induced either by Thorotrast or two large doses of colloidal carbon.

The results show that the stimulant effect of Arvin on the RES was still present in blocked animals.

*Opsonization of carbon with serum from Arvin-treated rabbits (Table 4)*

In order to determine whether the stimulant action of Arvin on the RES involves serum opsonins, colloidal carbon was incubated with serum collected from Arvin (1 u./kg) or saline-treated animals at 4 h or 10 min after injection.

TABLE 1. *Effect of a single dose of Arvin on rate of carbon clearance*

| Dose of Arvin u./kg | Pretreatment time | No. of animals per group | Mean intravascular half-time (min)* | |
|---|---|---|---|---|
| | | | Arvin | Saline |
| 0·01 i.v. | 4 h | 2 | 5·88 | 8·12 |
| 0·05 i.v. | 4 h | 4 | 3·81±3·36 | 9·18±2·11 |
| 0·1 i.v. | 4 h | 4 | 2·75±0·87 | 13·0±4·96 |
| 1·0 i.v. | *ca.* 1 min | 2 | 2·0 | 7·75 |
| 1·0 i.v. | 10 min | 2 | 2·0 | 10·0 |
| 1·0 i.v. | 30 min | 2 | 2·0 | 7·65 |
| 1·0 i.v. | 1 h | 2 | 2·65 | 10·5 |
| 1·0 i.v. | 4 h | 8 | 3·75±1·76 | 15·4±7·10 |
| 1·0 i.v. | 26 h | 2 | 5·10 | 11·25 |
| 1·0 i.v. | 48 h | 2 | 6·80 | 9·85 |
| 1·0 i.v. | 76 h | 2 | 10·30 | 11·95 |
| 10·0 i.m. | 2 h | 2 | 1·55 | 12·05 |
| 10·0 i.m. | 4 h | 2 | 2·90 | 9·20 |
| 10·0 i.m. | 24 h | 4 | 4·65±2·15 | 7·58±9·20 |
| 10·0 i.m. | 48 h | 4 | 5·27±3·67 | 9·70±4·70 |
| 10·0 i.m. | 72 h | 2 | 10·25 | 9·50 |
| 1·0 i.v. daily for 3 days | Last dose 3 h | 2 | 3·2 | 9·4 |
| 10·0 i.m. daily for 4 days | Last dose 3 h | 2 | 3·05 | 10·15 |

* Where four or more animals per group have been used the mean is given ±S.D.

TABLE 2. *Effect of anti-Arvin serum on the stimulant action of Arvin on carbon clearance rate*

| Pretreatment of rabbit before test. Dose in u./kg i.v. | Pretreatment period (h) | No of animals per group | Mean intravascular half-time (min) |
|---|---|---|---|
| 1 Arvin+2 Antiserum | 4 | 5 | 1·39±0·53 |
| Saline | 4 | 2 | 7·15 |
| 1·1 Arvin+2 Antiserum | 4 | 2 | 10·75 |
| Saline+2 Antiserum | 4 | 6 | 8·9±3·6 |

TABLE 3. *Effect of Arvin on carbon clearance rate in rabbits with RES blockade*

| Blocking agent | Time before test (h) | Arvin 1 u./kg or saline i.v. | | | |
|---|---|---|---|---|---|
| | | | Time before test (h) | No. of animals per group | Mean intravascular half-time (min) |
| Thorotrast 3 ml/kg | 3 | — | – | 2 | 29·00 |
| Saline | 3 | — | – | 2 | 11·35 |
| Thorotrast 3 ml/kg | 4 | Arvin | 3 | 4 | 3·17±2·01 |
| Thorotrast 3 ml/kg | 4 | Saline | 3 | 4 | 28·5±4·69 |
| Carbon 320 mg/kg | 4 and 28 | — | – | 2 | 29·50 |
| Saline | 4 and 28 | — | – | 2 | 4·00 |
| Carbon 320 mg/kg | 4 and 28 | Arvin | 3 | 2 | 3·50 |
| Carbon 320 mg/kg | 4 and 28 | Saline | 3 | 2 | 38·50 |

Anti-Arvin serum was added and the mixture was then injected into fresh rabbits and the clearance rate determined. Results show that whereas serum collected 10 min after Arvin had no effect, that collected at 4 h enhanced the rate of carbon clearance.

### *Effect of opsonization of carbon in RES blocked animals*

The rate of phagocytosis of serum-treated carbon was also measured in animals with RES blockade induced by Thorotrast or carbon. The results in Table 5 show that the enhancement of phagocytosis by 4 h serum from an Arvin-treated rabbit which is seen in a normal rabbit is not seen in a rabbit with RES blockade.

### *Effect of fibrinogen or fibrin degradation products on carbon clearance*

When fibrinogen is clotted by Arvin, the fibrinopeptides which are released are fibrinopeptides A and AP, rather than fibrinopeptides A, AP and B, as is the case with thrombin (Ewart *et al.*, 1969). The fibrin-like material which is formed *in vivo* by the action of Arvin on fibrinogen is presumably lysed, either by plasmin in the circulation, or by proteolytic enzymes in the cells of the RES. Since either the unusual fibrinopeptides, or the unusual fibrin degradation products, produced by Arvin might be responsible for the stimulant action of Arvin on the RES, we prepared samples of each, *in vitro*, and examined their effects on the rate of carbon clearance. The results in Table 6 show that neither of these preparations affected the rate of carbon clearance.

TABLE 4. *Effect of pre-opsonization on carbon clearance rate in rabbits*

| Opsonization of carbon before injection | No of animals per group | Mean intravascular half-time (min) |
|---|---|---|
| Test serum 4 h sample | 4 | 6·25±3·30 |
| Control serum 4 h sample | 4 | 20·94±4·20 |
| Test serum 10 min sample | 2 | 8·25 |
| Control serum 10 min sample | 2 | 10·5 |

TABLE 5. *Effect of pre-opsonization on carbon clearance rate in rabbits with RES blockade*

| Blocking agent i.v. | Pretreatment time (h) | Arvin serum or control serum | No. of animals per group | Mean intravascular half-time (min) |
|---|---|---|---|---|
| Carbon 320 mg/kg | 7 and 31 | Control | 2 | 40 |
| Carbon 320 mg/kg | 7 and 31 | Arvin | 2 | 40 |
| Thorotrast 3 ml/kg | 4 | Control | 2 | 20 |
| Thorotrast 3 ml/kg | 4 | Arvin | 2 | 15 |

TABLE 6. *Effect of Arvin-fibrinopeptides and Arvin-fibrin degradation products (FDP) on the rate of carbon clearance*

| Treatment of rabbit | Pretreatment period | No of animals per group | Mean intravascular half-time (min) |
|---|---|---|---|
| 5 ml/kg i.v. fibrinopeptides | 10 min | 2 | 11·5 |
| 5 ml/kg i.v. fibrinopeptides | 20 min | 2 | 9·4 |
| 5 ml/kg i.v. fibrinopeptide control | 20 min | 2 | 10·7 |
| 5 ml/kg i.v. fibrinopeptides | 4 h | 2 | 6·50 |
| 5 ml/kg i.v. fibrinopeptide control | 4 h | 2 | 7·00 |
| 5 ml/kg i.v. saline | 4 h | 2 | 7·20 |
| 2 ml/kg i.v. FDP | 4 h | 2 | 8·25 |
| 2 ml/kg i.v. FDP control | 4 h | 2 | 6·00 |

*Effect of a low dose of ovomucoid or bovine serum albumin on carbon clearance (Table 7)*

That the stimulant effect of Arvin on the RES was not a non-specific effect of a small amount of protein was shown by measuring the rate of carbon clearance after ovomucoid, a protein with a similar molecular weight and carbohydrate content to Arvin, and after bovine serum albumin, a protein which has similar gel filtration characteristics to Arvin. The results show that neither of these proteins affected the rate of carbon clearance.

*Post-mortem results*

In several cases the rabbits were killed with pentobarbitone and a visual examination carried out. In the case of the saline-treated rabbits, the liver and spleen were heavily blackened, and the lungs either lightly blackened or not blackened at all, whereas in the case of the Arvin-treated rabbits the liver, lungs and spleen were all heavily blackened. Haematoxylin and eosin stained sections of lung were examined by Dr. Mawdesley-Thomas of the Huntingdon Research Centre. These showed the location of carbon particles in the lung to be mainly intracellular.

## Discussion

The process of defibrination by crude *Agkistrodon rhodostoma* venom involves the production of microclots which are subsequently lysed in blood or, after phagocytosis, by cells of the RES (Regoeczi *et al.*, 1966). Arvin itself is possibly taken up and metabolized by reticulo-endothelial cells (Regoeczi & Bell, 1969) and these facts suggest that an effect of Arvin on RES activity might be expected.

Esnouf & Marshall (1968) studied the uptake of radioactive colloidal gold in dogs and found no change in RES activity after the administration of crude *Agkistrodon rhodostoma* venom. In contrast, our experiments show that Arvin exerted a powerful stimulant effect on the RES of rabbits at very low dose levels, the doses of 0·05–1·0 u./kg being equivalent to 0·1–2·0 $\mu$g protein/kg.

Perhaps the difference between our results and those of Esnouf & Marshall (1968) is due to the use of a purified venom fraction on the one hand and crude whole venom on the other. It may also be due to the use of different colloidal material to measure RE activity. However, since there is also a marked difference in duration of Arvin-induced defibrination between rabbits and dogs (Ashford *et al.*, 1968) a more likely explanation is species difference. The duration of defibrination in rabbits is relatively short compared with that in dogs, and in view of the possibility that Arvin is taken up and degraded by RE cells (Regoeczi & Bell, 1969), the duration of effect may be linked with RE activity and the degree to which this is

TABLE 7. *Effect of ovomucoid and of bovine serum albumin on the rate of carbon clearance*

| Treatment of rabbit before test | Pretreatment period (h) | No of animals per group | Mean intravascular half-time (min) |
|---|---|---|---|
| Ovomucoid 2 $\mu$g/kg i.v. | 4 | 2 | 7·20 |
| Bovine serum albumin 2 $\mu$g/kg i.v. | 4 | 2 | 10·20 |
| Saline i.v. | 4 | 2 | 6·00 |

stimulated by the drug. Arvin may promote its own destruction in rabbits but not in dogs.

In trying to decide on a mechanism of action, several results should be considered. The onset of effect following an intravenous injection of Arvin was very rapid, maximum stimulation being achieved in less than 1 min. Serum collected at 10 min after Arvin and incubated with carbon *in vitro* did not stimulate the subsequent intravascular clearance of the carbon, whereas serum collected at 4 h did stimulate clearance—that is, it had an opsonizing effect. The action of Arvin was still present, but the stimulant effect of 4 h serum was prevented in RES blocked animals. It is known that RES blockade may be caused either by saturation of phagocytic cells by the blocking agent (Stuart *et al.*, 1960 ; Biozzi, Stiffel, Halpern & Mouton, 1963) or by a depletion of serum opsonins, the role of these substances in the phagocytosis of inert colloids being well documented (Jenkin & Rowley, 1961 ; Normann & Benditt, 1965 ; Saba & DiLuzio, 1968). These facts suggest a dual mode of action—an initial direct effect of Arvin on phagocytic cells and a more delayed effect by serum factors on the colloidal particles.

The possibility that fibrinopeptides or fibrin degradation products might be responsible for the RE stimulant effect of Arvin was examined, but we found no evidence to support this view.

The serum factor is unlikely to be Arvin itself, both because of the presence of an excess of anti-Arvin serum and because serum collected 10 min after injection did not stimulate carbon uptake, although the level of Arvin at this time would be higher than that 4 h later.

Regoeczi & Bell (1969) described the release of Arvin degradation products from RES cells and it is conceivable that these constitute the serum-borne stimulant factor. However, the fact that RES blockade prevented the opsonizing action of 4 h serum suggests an action via naturally occurring opsonins.

The effect of Arvin in increasing carbon uptake by the lung is of particular interest. Intravascular coagulation induced in rats by thrombin, thromboplastin or shellac, a thromboplastin releasing agent, markedly increases the rate of removal of carbon from the circulation (Halpern, Benacerraf & Biozzi, 1953). These authors suggested that this effect was due to intravascular fibrin formation, and showed that much carbon was retained in the pulmonary vessels.

Although Arvin causes intravascular coagulation this property may be dissociated from its action on carbon clearance rate for several reasons. First, the RES stimulant effect of Arvin was marked at an intravenous dose of 0·1 u./kg although this dose of Arvin in the rabbit has a negligible effect on fibrinogen (unpublished observations). Second, neither the fibrinopeptides released from fibrinogen by Arvin, nor the degradation products formed by the lysis of fibrin had any effect on the rate of carbon clearance, and finally, although neutralization of 1 u. of Arvin by 2 u. of antiserum completely blocked the usual defibrinating effect of this dose, it did not prevent stimulation of carbon clearance rate.

The RES stimulation induced by 1 u. Arvin/kg was still present 48 h after the injection, although at this time plasma fibrinogen levels are returning to normal (Ashford *et al.*, 1968), and presumably intravascular coagulation has ceased.

## REFERENCES


ASHFORD, A., ROSS, J. W. & SOUTHGATE, P. (1968). Pharmacology and toxicology of a defibrinating substance from Malayan pit viper venom. *Lancet*, **1**, 486–489.

BELL, W. R., PITNEY, W. R., OAKLEY, C. M. & GOODWIN, J. F. (1968). Therapeutic defibrination in the treatment of thrombotic disease. *Lancet*, **1**, 490–493.

BIOZZI, G., BENACERRAF, B. & HALPERN, B. N. (1953). Quantitative study of granulopectic activity of the reticulo-endothelial system. II. Study of the kinetics of the granulopectic activity of the R.E.S. in relation to the dose of carbon injected. *Br. J. exp. Path.*, **34**, 441–457.

BIOZZI, G., STIFFEL, C., HALPERN, B. N. & MOUTON, D. (1963). Lack of action of serum opsonins in phagocytosis of inert particles by cells of reticulo-endothelial system. *Proc. Soc. exp. Biol. Med.*, **112**, 1017–1020.

ESNOUF, M. P. & MARSHALL, R. (1968). The effect of blockade of the reticulo-endothelial system and of hypotension on the response of dogs to *Ancistrodon rhodostoma* venom. *Clin. Sci.*, **35**, 261–272.

ESNOUF, M. P. & TUNNAH, G. W. (1967). The isolation and properties of the thrombin-like activity from *Ancistrodon rhodostoma* venom. *Br. J. Haemat.*, **13**, 581–590.

EWART, M. R., HATTON, M. W. C., BASFORD, J. M. & DODGSON, J. S. (1969). The proteolytic action of Arvin on human fibrinogen. *Biochem. J.*, in the Press.

HALPERN, B. N., BENACERRAF, B. & BIOZZI, G. (1953). Quantitative study of the granulopectic activity of the reticulo-endothelial system. I. Effect of the ingredients present in Indian Ink and of substances affecting blood clotting *in vivo* on the fate of carbon particles administered intravenously in rats, mice and rabbits. *Br. J. exp. Path.*, **34**, 426–440.

JENKIN, C. R. & ROWLEY, D. (1961). The role of opsonins in the clearance of living and inert particles by cells of the reticulo-endothelial system. *J. exp. Med.*, **114**, 363–374.

MARSHALL, R. & ESNOUF, M. P. (1968). The effect of *Ancistrodon rhodostoma* venom in the dog. *Clin. Sci.*, **35**, 251–259.

NORMANN, S. J. & BENDITT, E. P. (1965). Function of the reticulo-endothelial system. I. Phenomenon of carbon clearance inhibition. II. Participation of a serum factor in carbon clearance. *J. exp. Med.*, **122**, 693–719.

REGOECZI, E. & BELL, W. R. (1969). *In vivo* behaviour of coagulant enzyme from *Agkistrodon rhodostoma* venom: Studies using $^{131}$I-"Arvin". *Br. J. Haemat.*, **16**, 573–587.

REGOECZI, E., GERGELY, J. & MCFARLANE, A. S. (1966). *In vivo* effects of *Agkistrodon rhodostoma* venom: Studies with fibrinogen $^{131}$I. *J. clin. Invest.*, **45**, 1202–1212.

REID, H. A. & CHAN, K. E. (1968). The paradox in therapeutic defibrination. *Lancet*, **1**, 485–486.

REID, H. A., CHAN, K. E. & THEAN, P. C. (1963). Prolonged coagulation defect in Malayan viper bite. *Lancet*, **1**, 621–626.

SABA, T. M. & DILUZIO, N. R. (1968). Involvement of the opsonic system in starvation induced depression of the reticulo-endothelial system. *Proc. Soc. exp. Biol. Med.*, **128**, 869–875.

SHARP, A. A., WARREN, B. A., PAXTON, A. M. & ALLINGTON, M. J. (1968). Anticoagulant therapy with a purified fraction of Malayan pit viper venom. *Lancet*, **1**, 493–499.

STUART, A. E., BIOZZI, G., STIFFEL, C., HALPERN, B. N. & MOUTON, D. (1960). The stimulation and depression of reticulo-endothelial phagocytic function by simple lipids. *Br. J. exp. Path.*, **61**, 599–604.


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