non-aqueous solvents. However, its solubility in methanol is abnormally high. This high solubility can be attributed to some sort of solute-solvent interaction. Co-laurate, when dissolved in methanol, shifts the equilibrium $CoL_2 \rightleftarrows Co^{2+} + 2L$-towards the right due to the removal of Co^{2+} ions by methanol resulting in high solubility. Luz[11–13] recently reported the existence of a hexamethanol complex $[Co(MeOH)_6]^{2+}$, in methanol solution of Co^{2+} ions containing different amounts of chloride ions, at very low temperatures and the existence of the equilibrium $[Co(MeOH)_6]^{2+} \rightleftarrows [Co(MeOH)_5\ Cl]^+$ at higher temperatures. The relatively higher solubility of Co-laurate in methanol may be explained in terms of the existence of such cobalt complexes as $[Co(MeOH)_6]^{2+}$ and $[Co(MeOH)_5L]^+$ (L = Laurate).

It has been observed that the solubility of cobalt soap in benzene increases abnormally by adding small amounts of methanol. Solubility of Co-laurate in 1:9 methanol-benzene mixture is about 1000 times that of the cobalt soap in benzene.

Further work on the effect of the additives on the solubility of Co-laurate is in progress.

Solubility of cobalt laurate in water and non-aqueous solvents at 30 °C.

Solvents	Solubility · 10^5 (M/l)
Water	2.295 ± 0.094
Dimethyl formamide	1.992 ± 0.022
Methyl ethyl ketone	2.121 ± 0.025
Acetone	2.396 ± 0.025
Ethanol abs.	3.943 ± 0.061
Methanol abs.	604.25 ± 3.01
Benzene	7.985 ± 0.087
Cyclohexane	4.435 ± 0.058
Toluene	3.967 ± 0.074
Carbon tetrachloride	1.159 ± 0.029

Zusammenfassung. Die bisher unbekannte Löslichkeit von Kobaltlaurat wurde mit Hilfe der Tracer-Technik unter Verwendung von ^{58}Co bestimmt. Die Löslichkeit in Wasser und organischen Lösungsmitteln (mit Ausnahme von Methanol) ist sehr gering. Die relativ grosse Löslichkeit in Methanol wird auf Bildung von Co^{2+}-Methanol-Komplexen zurückgeführt.

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[11] Z. Luz, J. Chim. phys. *41*, 1748 (1964).
[12] Z. Luz, J. Chim. phys. *41*, 1756 (1964).
[13] Z. Luz and S. MEIBOOM, J. Chim. phys. *40*, 1058 (1964).

Adherence of Opsonized Lymphocytes to Macrophage Cultures

Adherence or phagocytosis of opsonized particles by macrophages is well known and has been used to detect antibodies to bacteria[1,2], erythrocytes[3,4] and rather less commonly nucleated cells[5,6]. Phagocytosis of tumour cells[6] by macrophages has been demonstrated in the presence of immune serum. Since the immune response to a transplantable tumour[7] may be vigorous it seemed worthwhile to determine whether immunophagocytosis might still be useful for the detection of iso-antibody when induced by the injection of normal cells. 2 advantages of this technique are that results can be read easily and the preparations may be stored as permanent records. The sensitivity of immunophagocytosis was compared directly with agglutination[8] and cytotoxic tests[9] for iso-antibodies.

The present experiment utilized cultures of extended macrophages (24 h old), and normal lymphocytes were used for interaction with macrophages in the presence of iso-antibody. An immune serum was prepared by injecting mice with lymphocytes from a different strain. This serum was incubated overnight with tissue culture preparations of normal macrophages. When normal lymphocytes from the donor animals were added to these cells a marked degree of adherence and phagocytosis was observed. Normal serum failed to give any significant reaction. 30 CBA mice (Animal Suppliers, London) were given $100 \cdot 10^6$ lymphocytes by the i.p. route every 3 days for a total of 6 injections. The lymphocytes were obtained by grinding axillary, inguinal, mesenteric and submandibular lymph nodes in a hand operated homogenizer with a $^1/_{32}$ inch clearance between the plunger and the barrel. The animals were bled 7 days after the last injection and the serum sterilized by passage through a Millipore Swinnex filter. It was stored at 4 °C and used the following day. The cytotoxic test[9] for demonstration of mouse antibody was used to measure the titre of the anti-lymphocytic serum. The lymphocytes were taken from the mesenteric, inguinal, axillary and submaxillary glands of normal C57B1 mice. The lymph nodes were teased into a suspension and injected through a No. 19 needle. The macrophage monolayers were prepared from normal CBA mice[10]. The animals were killed, the abdominal cavity rinsed out with 2 ml of medium 199 (Glaxo) containing 5 units of heparin/ml and the cell count adjusted to $1 \cdot 10^6$/ml. Sufficient normal or immune CBA

[1] G. BIOZZI, C. STIFFEL, L. LE MINOR, D. MOUTON and Y. BOUTHILLIER, Annls Inst. Pasteur, Paris *105*, 635 (1963).
[2] S. SLOPEK, K. GRYBEK-HRYNCEWICZ, J. LADOSZ and K. KUBIS, Archs Immun. Ther. Exp. *13*, 302 (1965).
[3] C.-S. WRIGHT, M. C. DODD, N. G. BRANDT, S. M. ELLIOTT and J. H. BASS, J. Lab. & clin. Med. *41*, 169 (1953).
[4] A. E. STUART and R. A. CUMMING, Vox Sang, in press (1967).
[5] P. MIESCHER, Dt. med. Wschr. *83*, 216 (1953).
[6] B. BENNET, L. J. OLD and E. A. BOYSE, Transplantation *2*, 183 (1964).
[7] A. M. EL. HASSAN and A. E. STUART, Br. J. Cancer, *19*, 343 (1965).
[8] D. B. AMOS, Br. J. exp. Path. *34*, 464 (1953).
[9] E. A. BOYSE, L. J. OLD and I. CHOUROULINKOV, Meth. med. Res. *10*, 39 (1964).
[10] J. L. WHITBY and D. ROWLEY, Br. J. exp. Path. *40*, 357 (1959).

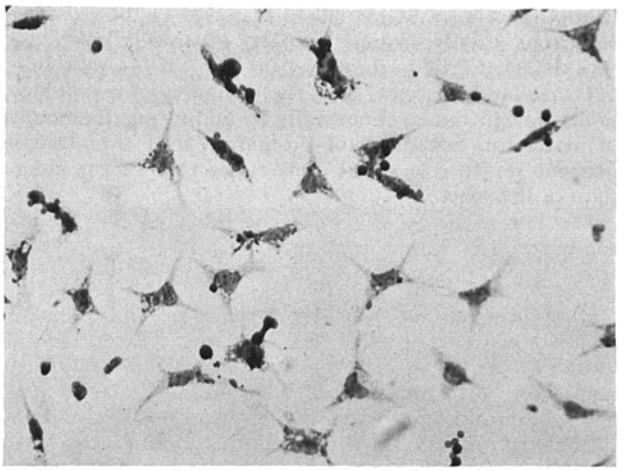

Fig. 1. CBA macrophage monolayer, normal CBA serum and C57B1 lymphocytes. Leishman stain × 350.

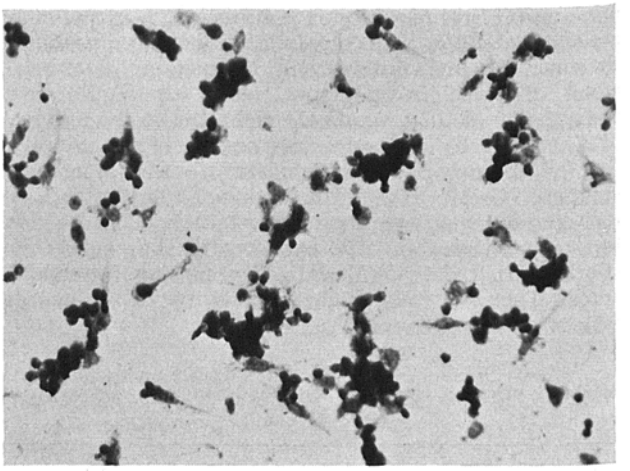

Fig. 2. CBA macrophage monolayer, immune CBA serum and C57B1 lymphocytes. Leishman stain × 350.

serum was added to give a final concentration of 15% and 1.5 ml volumes were added to rimless Pyrex tubes containing rectangular glass coverslips 19 · 6 mm. The tubes were incubated overnight at 37 °C at an angle of 5° to the horizontal. A suspension of normal C57B1 lymphocytes was prepared which contained between $25 \cdot 10^6$–$35 \cdot 10^6$ cells/ml. 0.2 ml volumes were added to the monolayer, shaken gently and incubated for a further 1 h. The tubes were rinsed thoroughly with 0.85% sodium chloride solution, fixed in methanol and stained by Leishman's method.

In a second experiment designed to determine whether macrophages from outbred animals could be substituted for CBA cells, similar monolayers were prepared from Tuck's T.O. strain and grown in 15% serum from that strain. The C57B1 lymphocytes were incubated for 30 min with T.O. serum, normal CBA serum and immune CBA serum. The suspensions were then added to the monolayers for 1 h when they were fixed and stained. All experiments were carried out in duplicate and each experiment has been done twice.

The control cultures which contained normal serum showed well-defined macrophages and only small numbers of lymphocytes (Table). The effect of adding immune serum was clearly visible under the low power of the microscope which revealed large darkly stained agglomerations of cells (Figures 1 and 2). These were composed of small round cells which were firmly adherent to the macrophages. In some fields rosettes were conspicuous; these had a mulberry appearance and were formed from numerous lymphocytes adherent to a single macrophage. Sometimes coronet forms were seen in which the phagocytes had a halo of small lymphocytes and in many preparations there was a marked degree of phagocytosis. Phagocytosis is not always easy to distinguish from surface sticking, but certainly some lymphocytic nuclei were within vacuoles in the cytoplasm of the macrophage. The immune serum was active to a titre of over $^1/_{32}$ and less than $^1/_{64}$ by the tissue culture method. Agglutination titres were in the region of $^1/_4$ but spontaneous clumping made interpretation difficult. The cytotoxic test with Trypan blue gave titres of $^1/_2$–$^1/_4$. These results show that the discriminatory activity of the macrophage is not without a moderate degree of sensitivity.

The number of lymphocytes added to the cultures is critical; $10 \cdot 10^6$ is too many and the optimal numbers are between $2 \cdot 10^6$ and $5 \cdot 10^6$ cells. The other variables are the time required for opsonization with iso-antibody and the duration of incubation of sensitized cells with the macrophage cultures. As a general rule the best results were obtained using long sensitization times and short incubation with the cultures. It is curious that allogeneic macrophages grown in their own serum were also effective in 'recognizing' normal C57B1 lymphocytes sensitized by immune CBA serum. Whilst this simplifies the technique it is not yet possible to say whether or not the sensitivity of the system has been reduced. It is important to realize that the system described here does not necessarily involve histocompatibility antigens although it is hoped that the method will extend the scope of in vitro studies on the pathology of the homograft reaction.

Effect of isogeneic and allogeneic macrophages on immuno-phagocytosis of lymphocytes

Source of serum	Source of macrophages	Source of donor cells	Result
Normal CBA	CBA	C57B1	Negative
	CBA	CBA	Negative
	T.O.	C57B1	Negative
Immune CBA	CBA	C57B1	Positive
	CBA	CBA	Negative
	T.O.	C57B1	Positive
Normal T.O.	T.O.	C57B1	Negative

Résumé. Un sérum du type «iso-immune» a été préparé en injectant des cellules lymphoides C57B1 dans le sang de souris CBA. Ce sérum a été ensuite titré par agglutination, par la méthode du «Trypan bleu» et par l'opsonophagocytose. On constata que l'opsonophagocytose donne le titre le plus élevé et a l'avantage d'offrir des préparations permanentes.

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