gress and among certain public and private groups. These groups include labor organizations and publicly and cooperatively owned power systems. The opposition in most instances is directed against hasty and ill-considered action, and the timing of any action, rather than against the proposition that private enterprise should participate in the development of nuclear power. Many are of the opinion that there is no need for drastic legislative revisions at this time, that the AEC should and will support the necessary programs. In view of the abundant evidence to the effect that military interest is a prerequisite if a project hopes to obtain and retain financial support from public sources, I doubt that this approach is realistic.

I would not want to predict what actions, if any, will eventually be taken in the legislative field. However, I would venture to say that the formulation and enactment of specific revisions with regard to the major items of ownership of fissionable material, production plants, and patents which will be satisfactory to private enterprise and at the same time protect the public's interests will take a long time. At best, we cannot expect any firm commitments for power-only projects supported wholly by private risk capital until satisfactory legislative changes are consummated.

In concluding, I would like to specify a little more precisely the type of program called for in my title. First, it should have a single purpose. It should be directed solely toward the production of nuclear power for civilian uses—both short and long range. Second, the program and its objectives should be assured of continuing financial support, barring national emergencies or adverse technical developments. Third, it should require a minimum of associated administrative policy and legislative problems which would cause delays in schedule.

I would like to reemphasize what I said at the beginning. Whether or not this country establishes a strong single-purpose program to develop a practical nuclear power plant in the near future is not of vital importance to our general welfare or to our national security. So far as our own direct interests in nuclear power are concerned, it seems unlikely that a delay of ten years or more in starting a serious development program will have any serious effect.

However, the report of the President's Materials Policy Commission emphasized our dependence on other friendly nations for raw materials, uranium certainly not the least important of these, that add immeasurably to our own industrial and military strength. The technical and financial resources at our disposal would enable us, with relatively little extra effort, to develop a technology which shows promise of being of significant short-term importance to some of these countries. This is an opportunity to return "strength for strength." It is an opportunity that will not wait ten years, nor perhaps even half that length of time. It is rapidly slipping through our hands while other countries, presumably Russia included, are moving ahead with decisiveness.

The Immune-Adherence Phenomenon

An Immunologically Specific Reaction Between Microorganisms and Erythrocytes Leading to Enhanced Phagocytosis

Robert A. Nelson, Jr.[1]

Department of Microbiology, The Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland, and
Bacteriology Division, Naval Medical Research Institute, Bethesda, Maryland

IN STUDIES designed to demonstrate phagocytosis of virulent *Treponema pallidum in vitro* (*1, 2*), observations on control preparations led to a recognition of an immunologically specific reaction between normal human erythrocytes and treponemes sensitized with antibody from syphilis serum. The reaction required a heat labile substance in normal serum, presumably complement (C′). Although not definitive, the experiments suggested that this reaction was an essential precursor to phagocytosis of the treponemes by human leucocytes.

The present experiments provide additional data on the reaction of *T. pallidum* with erythrocytes, and demonstrate that a similar reaction occurs with other microorganisms, e.g., *Diplococcus pneumoniae, Shigella paradysenteriae, Salmonella typhi, Micrococcus aureus,* and *Mycobacterium tuberculosis.* Further, it is shown that the union of sensitized organisms with erythrocytes, hereafter termed the immune-adherence phenomenon, leads to an enhancement of phagocytosis.

Experiments with *T. pallidum*

Requirement for Antibody (Table 1). Treponemes were isolated from testicular syphilomas of rabbits irradiated with 600 r prior to infection as previously

[1] Howard Hughes Foundation fellow in medical research.

described (*1–3*). Seven-tenths milliliter of a suspension[2] containing approximately 10^7 organisms/ml was mixed with 0.1 ml of: (*a*) ultrafiltrate of ox blood serum as a protein-free control; (*b*) normal rabbit serum; and (*c*) varying dilutions of syphilitic rabbit serum. After 30 min at 37° C, 0.2 ml of normal human blood was added to each mixture. After an additional 30 min the blood cells were removed by centrifugation at low speed (500 rpm, 5 min). The number of treponemes in the fluid phase was counted by darkfield microscopic examination of 0.01 ml of supernatant fluid placed under a 22-mm^2 coverslip at 675 × magnification.

TABLE 1

ESSENTIAL ROLE OF ANTIBODY IN REACTION OF *T. pallidum* WITH NORMAL HUMAN WHOLE BLOOD

Material added to treponemes prior to addition of blood	Initial serum dilution	Treponemes in fluid phase after incubation with whole blood for 30 min*
Serum ultrafiltrate	Undil.	92
Normal serum	Undil.	96
Syphilis serum	Undil.	0
Syphilis serum	1 : 5	4
Syphilis serum	1 : 25	34
Syphilis serum	1 : 125	86
Syphilis serum	1 : 625	86

* Number of treponemes per 10 fields in suspension not exposed to serum or blood cells, but brought to volume with saline only, 98.

While normal rabbit serum and human whole blood were inactive in causing disappearance of the treponemes from the fluid phase, syphilis serum was highly active in dilutions up to 1:25. The specificity of this reaction for antibody in syphilis serum was established by examination of 385 human sera (*4*). Reactivity of the sera in inducing clearing of the fluid phase corresponded with the clinical diagnosis of syphilis and with results of the treponemal immobilization test in all except five instances.

Necessity for C′ (Table 2). Mixtures containing 0.6 ml of treponemal suspension, 0.1 ml of syphilis serum, and 0.2 ml of washed human blood cells, were mixed with 0.1 ml of varying dilutions of fresh serum as a source of C′ from each of three species: human, guinea pig, and rabbit. After 30 min at 37° C, the mixtures were centrifuged and the treponemes in the fluid phase were counted by darkfield microscopy.

Greater than 90 per cent of the treponemes disappeared from the fluid phase of mixtures with undiluted C′ of the three species. No disappearance occurred in controls containing only heated serum. On the basis of the dilution required for disappearance of 50 per cent of the treponemes, human (1:34) and guinea pig (1:36) C′ were approximately equal in

[2] Relatively dilute suspensions of all microorganisms have been employed in this study in order to avoid agglutination. With organisms highly susceptible to agglutination, immune serum was used at a dilution exceeding that which caused agglutination.

TABLE 2

REACTIVITY OF THREE SPECIES* OF C′†

Reciprocal of C′ dilution‡	1	4	16	64	256	Heated serum only, undiluted
Human serum	2	32	80	146	235	215
Guinea pig serum	2	18	74	145	255	250
Rabbit serum	14	62	175	246	250	245

* The 3 samples of serum used as a source of C′ were assayed previously with nonsensitized treponemes and found to be free of specific treponemal antibody.

† Number of treponemes per 20 fields in fluid phase of mixtures containing sensitized treponemes, washed human blood cells, and dilutions of fresh serum (C′). Treponemes were sensitized 60 min at 37° C with syphilis serum, previously heat-inactivated to remove C′.

‡ Fresh serum diluted in heated (56°, 60 min) serum.

reactivity, whereas rabbit (1:7) C′ was weakly reactive. By contrast, the hemolytic titers of these same sera, measured by the technique of Mayer *et al.* (*5*), were as follows: human, 24; guinea pig, 195; and, rabbit, 10.

Later experiments with pneumococci and erythrocytes in which guinea pig serum was absorbed with a washed specific precipitate of a heterologous antigen-antibody system have indicated that the heat labile substance is C′.

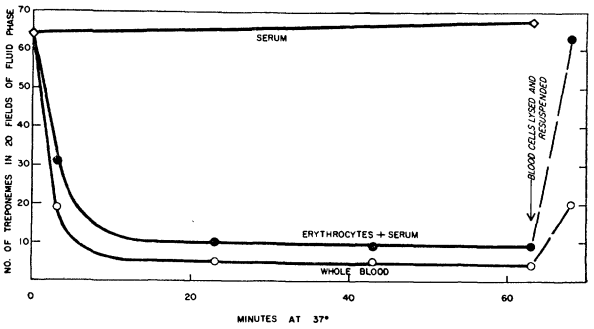

FIG. 1. The rate of clearing of the fluid phase induced by whole blood and by erythrocytes and C′. Recovery of the treponemes from the centrifugate of mixtures containing erythrocytes and C′.

Role of Erythrocytes (Fig. 1). Four mixtures were prepared containing 8 volumes of sensitized treponemes with 2 volumes of: (*a*) fresh human serum (C′) only; (*b*) defibrinated human blood; (*c*) washed human erythrocytes and C′; and (*d*) washed human leucocytes and C′. Samples were removed and centrifuged immediately after mixing, and thereafter at intervals of 20 min for 60 min. The numbers of treponemes in the fluid phase were counted by darkfield microscopy. At the end of the experiment the sedimented blood cells were lysed by alternative freezing and thawing to decrease refractility of the erythrocytes, and then resuspended to the volume of the original reaction mixture. Samples were removed for counting of the treponemes by darkfield microscopy.

Clearing of the fluid phase occurred equally in the mixture containing whole blood and in the mixture

containing washed erythrocytes and C′. No decrease in the number of organisms occurred in mixtures with C′ only, or with leucocytes and C′.

The explanation for the reactivity of erythrocytes became apparent when the sedimented red cells were resuspended at the end of the experiment. Essentially all the treponemes, i.e., 62 of the original 64, could be recovered, and the vast majority of these were adherent to the surface of the erythrocytes. It was obvious that the union of the organism with the erythrocyte, which required antibody and C′, had caused the disappearance of the treponemes from the fluid phase.

However, in mixtures containing whole blood, only 20 per cent of the organisms were recovered upon resuspension of the lysed blood cells. It is suggested that the difference between recovery from mixtures with erythrocytes and recovery from mixtures with whole blood represents the number of organisms phagocytized by leucocytes in the latter.

In experiments not shown here, the specific role of the erythrocyte in this reaction was indicated in that no clearing of the fluid phase and no microscopic evidence of adherence of treponemes was noted when erythrocytes were replaced by human platelets,[3] by fine particles of charcoal or magnesium silicate, or by suspensions of *Candida albicans*. Furthermore, sensitized treponemes did not react with washed sheep erythrocytes, and were only slightly reactive with washed rabbit erythrocytes, when the assays were performed under the conditions outlined above.

Experiments with *D. pneumoniae*

Adherence to Erythrocytes (Table 3). Normal human blood was defibrinated by shaking with glass beads. Samples of erythrocytes and of leucocytes were prepared by the albumin flotation technique (7) and were restored to original concentration in fresh autologous serum. Heat-killed (56° C, 15 min) pneumococci, type 1, were washed in saline, and 0.5-ml samples were sensitized with 0.1 ml of rabbit pneumococcal antibody (1.6 μg antibody nitrogen) at a concentration slightly less than that which produced microscopic agglutination. Suspensions of sensitized organisms, as well as suspensions of nonsensitized controls, were mixed with the 0.1-ml samples of human blood cells as shown in the table and gently agitated at 37° C for 60 min. The blood cells were removed by low speed centrifugation and the numbers of organisms remaining in the fluid phase were determined by darkfield microscopy.

There were no decreases in mixtures without antibody. No decrease occurred in blood cell—free controls with antibody only or with antibody and C′. Approximately 98 per cent of the sensitized pneumococci were removed from the fluid phase by defibrinated whole blood. From classical concepts it might have been interpreted that this clearing of the fluid phase by whole blood was due to phagocytosis of the pneumococci by leucocytes. However, as in the experiments with *T. pallidum*, washed erythrocytes and C′ were quantitatively as reactive as whole blood in clearing the fluid phase.

[3] The lack of reactivity of platelets was confirmed in repeated assays, since it was in contrast to the results obtained by Rieckenberg with leptospira (6). The latter reaction, termed the *thrombocytobarin* phenomenon, consisted of the adhesion of platelets or of bacteria to leptospira in the presence of specific leptospiral antiserum.

TABLE 3

Reactivity of Blood Components in Clearing the Fluid Phase of Mixtures Containing Pneumococci and Antibody

Materials added to suspension of pneumococci	No. of pneumococci* in the fluid phase after 60 min at 37° C (per 10 fields)	
	Without antibody	With antibody
None	120	118
Serum only (C′)	Not measured	120
Whole Blood	108	2
Erythrocytes + C′	110	1
Leucocytes + C′	Not measured	140

* Chains of organisms were counted at 1 unit.

Despite the fact that phagocytosis was expected to occur, there was no decrease in the number of organisms in the fluid phase of the mixture containing leucocytes, antibody, and C′. It should be emphasized, however, that the experimental conditions were designed to minimize contact between organism and phagocyte, i.e., relatively dilute suspensions of both pneumococci and leucocytes were employed. The rationale for the experimental design will be discussed in detail in future publications but, in brief, the proportion of reagents was adjusted to simulate what is believed to be the natural events wherein an immune, or partially immune, host is exposed frequently to small numbers of pathogenic microorganisms that gain access to the body tissues or the blood stream. The conditions wherein large numbers of bacteria are in contact with large numbers of leucocytes, e.g., in abscesses, may involve different mechanisms of phagocytosis.

Upon darkfield examination of the resuspended centrifugate of the erythrocyte mixture, or directly of samples of this mixture without centrifugation, the majority of the pneumococci were found to be adherent to the surface of the erythrocytes, as in Fig. 2. No significant lysis of erythrocytes was noted. Agglu-

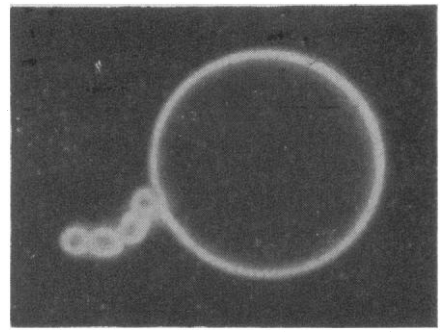

Fig. 2. Darkfield photomicrograph of *D. pneumoniae*, type 1, adherent to the surface of a normal human erythrocyte.

tination of the erythrocytes was not seen, although on occasions more than one erythrocyte was adherent to the same chain of pneumococci.

Enhancement of Phagocytosis (Table 4). Since no clearing of the fluid phase occurred with leucocytes in the preceding experiment, the procedure was repeated with larger numbers of leucocytes and a longer reaction time. Washed pneumococci, rabbit pneumococcal antibody, washed human erythrocytes, human C′, and leucocytes from an artificially produced exudate in the peritoneum of guinea pigs were employed as listed in the table. The degree of phagocytosis was determined on stained preparations of samples removed directly from the reaction mixture prior to centrifugation and recorded as the percentage of phagocytic cells containing pneumococci.[4] Since varying degrees of degeneration of pneumococci in phagocytes were noted in the 60- and 120-min samples, only the 30-min determination is recorded. Samples were removed at each time interval and were centrifuged for measurements of the number of organisms in the fluid phase.

There was no decrease in the number of pneumococci in the fluid phase of the antibody-free control at any of the times of sampling, and in 30 min only 4 per cent of the phagocytes contained organisms. Without erythrocytes, but with antibody and C′, there was a 20 per cent decrease in fluid phase count after 120 min, and 18 per cent of the leucocytes contained pneumococci. In the complete reaction mixture with antibody, C′, leucocytes and erythrocytes, 95 per cent of the organisms were removed from the fluid phase, and 60 per cent of the leucocytes exhibited phagocytosis at 30 min.[5]

Since antibody and C′ are essential for the immune-adherence reaction of microorganisms with normal erythrocytes, this phenomenon is differentiated from the absorption of soluble antigens, viruses, etc., on normal erythrocytes, which is postulated to be immunologically nonspecific. The latter absorption reaction, which often leads directly or indirectly to agglutination of the red cells, is stated to occur without, and in fact may be inhibited by, antibody, and does not require C′.

In studies on the localization of bacteria injected into immune animals, Rich (*8*) described tissue specimens in which the organisms were agglutinated and appeared to be adherent to tissue cells. The relationship is not clear between the observation of Rich and the findings of the present study for two reasons.

[4] Measurements of the percentage of phagocytes containing pneumococci were employed since the number of organisms within the phagocytes varied only from 1 to 4 in the majority of experiments.

[5] On repeated occasions samples of mixtures, similar to the complete reaction mixture described here, have been examined directly by darkfield microscopy. Numerous examples have been observed wherein leucocytes phagocytized pneumococci which were adherent to erythrocytes. These direct observations have proved of great interest in that varied maneuvers of the leucocyte have been seen, all of which, however, terminate in removal of the pneumococci by the leucocyte from the erythrocyte surface, leaving the erythrocyte free. Phagocytosis of both erythrocyte and the adherent organism has not been observed.

TABLE 4

ENHANCEMENT OF PHAGOCYTOSIS OF SENSITIZED PNEUMOCOCCI IN THE PRESENCE OF NORMAL HUMAN ERYTHROCYTES

Reaction mixtures*	No. of pneumococci in 10 fields of the fluid phase after			Percentage of leucocytes containing phagocytized pneumococci at 30 min
	30 min	60 min	120 min	
Antibody-free (Pn, RBC, WBC, C′)	80	80	84	4
Erythrocyte-free (Pn, Ab, WBC, C′)	78	78	60	18
Complete (Pn, Ab, RBC, WBC, C′)	21	12	4	60

* Volumes of reagents in 10 ml: pneumococci (Pn), 5 ml; approx. final concentration, 75×10^4/ml. Antibody (Ab), 1 ml; containing 16 μg antibody nitrogen. Leucocytes (WBC), 2 ml; approx. final concentration, 75×10^4/ml. Erythrocytes (RBC), 0.25 ml of packed cells. Complement (C′), 1 ml of fresh undiluted serum. The mixtures were incubated in silicone-treated (*Dry Film*, General Electric Corp.) tubes in a constant-speed rotator at 16 rpm at 37° C.

First, on repeated occasions in the experiments described here, organisms were seen in stained preparations to be directly adjacent to the various cells employed. Since this was noted in mixtures both with and without specific antibody and/or C′, it was adjudged to be an artefact incurred during drying and staining the preparation. To avoid this difficulty, fluid preparations were examined by darkfield or phase microscopy for the immune-adherence phenomenon. Thus, drying, fixing, and staining were unnecessary. Second, the present experiments indicate that certain other cellular elements will not substitute for the erythrocyte in the immune-adherence reaction, and, further, that only certain species of erythrocytes were capable of reactivity.

Under the experimental conditions of the present study in which limited numbers of organisms and of phagocytic cells are employed, the immune-adherence phenomenon leads to an enhancement of phagocytosis, and thus, the normal erythrocyte may function as an opsonic agent. The mechanisms by which the adherent microorganisms become more susceptible to phagocytosis are not defined. These may be similar to enhancement of phagocytosis of organisms in the absence of antibody wherein the phagocyte can trap the bacterium against certain surfaces, as demonstrated by Wood and associates (*9*). On the other hand, it is possible that substances from the erythrocyte surface may coat the microorganism and render it more susceptible to phagocytosis.

Since phagocytosis may occur without erythrocytes, classical techniques whereby concentrated suspensions of bacteria are mixed with suspensions containing large numbers of leucocytes might well mask the opsonizing role of the erythrocyte. Other techniques employing whole blood as a source of leucocytes and

in which stained preparations are examined at the completion of the experiment for organisms within leucocytes fail to reveal the events preceding engulfment, i.e., the potential opsonizing role of the erythrocyte. The present observations suggest the necessity for control of the number and species of erythrocytes in quantitative measurements of phagocytosis *in vitro*. Moreover, if antibody and C′ are present in the reaction mixture, it is obvious that phagocytosis alone is not measured by the technique of Maaløe (*10*), in which clearing of the supernate fluid from mixtures containing bacteria and whole blood is used as an end point. The present data demonstrate that in such measurements washed erythrocytes and C′ may be quantitatively as reactive as whole blood.

The observation that erythrocytes play a role in an immunologic reaction with organism, antibody, and C′ offers advantages to studies on the *in vivo* resistance of the host to infection, and also may lead to the development of clinical diagnostic procedures involving the use of preparations of erythrocytes and microorganisms for the detection of circulating antibody.

References

1. NELSON, R. A. *Presse méd.*, **60**, 1719 (1952).
2. ———. *Brit. J. Venereal Diseases*, **28**, 160 (1952).
3. NELSON, R. A., and MAYER, M. M. *J. Exptl. Med.*, **89**, 369 (1949).
4. NELSON, R. A. In preparation.
5. MAYER, M. M., *et al. J. Immunol.*, **59**, 195 (1948).
6. RIECKENBERG, H. *Z. Immunitätsforsch.*, **26**, 53 (1917).
7. VALLEE, B. L., HUGHES, W. L., and GIBSON, J. G. Supplement to *Blood*. Spec. Issue (1947).
8. RICH, A. *Bull. Johns Hopkins Hosp.*, **52**, 203 (1933).
9. WOOD, W. B. *Harvey Lectures, Ser.* **47** (1951, 1952).
10. MAALØE, O. *On the Relationship between Alexin and Opsonin*. Copenhagen: Munksgaard (1946).

News and Notes

Sixth International Congress for Microbiology

THE Congress was held in Rome Sept. 6–12, in the halls of the Città Universitaria. Concurrently six symposia on topics of general interest in microbiology were held in the adjacent Istituto Superiore di Sanità. Under the presidency of V. Puntoni, the organizers of the Congress were host to some 2000 delegates from 60 nations; the actual number of registrants, including guests and students, was undoubtedly much higher. In recognition of the Congress, the Italian Government issued a postage stamp that appropriately bore the picture of Agostino Bassi who, in 1834, showed for the first time that a microorganism was the causative agent of a disease in an animal. The Congress medal, presented to all delegates, was a very handsome relief of Franciscus Redi, done in heavy white metal from the design by Mistruzzi.

An elaborate program of entertainment was arranged for the members of the Congress and included a party in the beautiful garden of the Istituto Superiore di Sanità, a reception by civic officials at the Campidoglio, a symphony concert, and an excursion to the excavations at Hadrian's villa and to Tivoli. It was obvious to all that the organizers of the Congress, and especially the general secretary, E. Biocca, and his staff had striven to make every arrangement for the details of the Congress. That they succeeded nobly, was generally agreed.

Over 1000 communications were presented in the 4½ days devoted to the work of the Congress. The papers, covering an extremely wide range of topics, were arranged in 32 sections and subsections. Competition for attendance among sections covering closely related fields was intense and was aggravated by the competition from the some 60 papers of general interest presented concurrently among the six symposia. Most of the sections and subsections were devoted to subjects that have come to comprise the traditional structure of an international congress of microbiology. Many of the sections were well organized and featured arrays of papers of considerable general interest. However, one or two of the sections reflected the inability, or unwillingness, of the organizers of the Congress to reject papers offered to them. It was, indeed, a travesty on the large amount of substantial work contributed to the Congress that much of the press coverage was devoted to a few lurid accounts that ought not to have appeared on the program. The press coverage, in general, concerned itself with the spectacular and with the outlandish; although only the former might be classed as news for the average reader, a substantial level of science reporting would seem to be incumbent upon the metropolitan daily press.

Among the several pounds of printed matter handed each of the delegates were the abstracts of papers to the Congress (over 1000 abstracts contained in 3 large volumes) and a volume of abstracts of the symposia papers. It is especially noteworthy that the full texts of papers presented at the symposia were available in print while the Congress was still in session. The director of the Istituto Superiore di Sanità, Domenico Marotta, and his staff deserve commendation for getting the 6 sizeable volumes into print with such speed. The relatively low prices of the symposia volumes and the general interest of many of the papers contained therein will undoubtedly assure a wide demand for these books. The subjects of the symposia were: Bacterial Cytology, Microbial Metabolism, Nutrition and Growth Factors, Growth Inhibition and Chemotherapy, Biology of Actinomycetales, and Interaction of Viruses and Cells. It is impossible to single out for comment individual contributions from the 65 papers presented at the symposia or from the 1000 papers