water solution slowly moved the yellow band of color down the column. The alcohol solution was followed through the column by distilled water in order to flush the last of the alcohol through the resin bed. The alcohol–water eluate was concentrated at reduced pressure to 600 ml, and the sample set in the refrigerator overnight to allow precipitation to occur. The precipitate was collected and dried at 110° C. One and eight-tenths g of rutin was recovered from the column.

Paper partition chromatography of the recovered rutin revealed no fluorescent zone of quercetin in contrast to the easily detectable zone of quercetin in paper chromatograms of the original sample. The quercetin was subsequently recovered from the column by elution with 95% ethyl alcohol. Paper partition chromatography of the concentrated ethyl alcohol fraction revealed a small amount of rutin present along with the quercetin.

Preliminary studies indicate promising possibilities for expanding the applicability of ion exchange resins to flavonoid compounds through the use of ion exchange resins other than the Amberlite IRC-50. The flavonoids may also be adsorbed on the hydrogen form of Amberlite IRC-50 from solutions of their sodium, potassium, lead, or aluminum salts. Metal ion–hydrogen ion exchange occurs on the resin bed, and the adsorbed flavonoid may be subsequently eluted with alcohol.

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Alteration of Immunological Response in Malignancy: Decline of Proteus Agglutinin[1]

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In a recent study (*1*) focused on the course of nonspecific antibody in malignancy, we observed a fall in the *Proteus agglutinin* of chicken sera throughout the development of a Rous sarcoma. This finding, supported by literature indicating a lessened incidence of antibody in malignant disease, stimulated our interest and provoked our current investigation of antibodies to *Proteus* in human sera under normal and neoplastic conditions, of which we are now submitting a preliminary report.

Proteus agglutinin was selected as particularly convenient for our purpose since we found it to be quite common to normal adults. For all titrations described below the antigen used was a *Proteus* vaccine prepared from the OX19 strain according to our method described elsewhere (*1*). Before testing, the stock solution of antigen was diluted 10–15 times with saline, depending upon trial tests necessary to determine the dilution that would give agglutination with normal but not with cancer sera. As a normal control we used a pooled sample of sera from individuals with no apparent disease, and for a negative control, serum from a patient with a proved malignancy.

The actual test was done in small tubes (1.2 cm × 10.1 cm) stoppered with cork to prevent evaporation. To each tube containing 0.1 ml of inactivated serum (56° C for 30 min), whether undiluted or in dilution, we added 0.1 ml of our antigen. The tubes were incubated at room temperature and read with the aid of a binocular microscope (10 × and 23 ×) at several time intervals over a 2-hr period. We observed that the human sera which agglutinate with *Proteus* antigen give a strong reaction when used undiluted or diluted 1–10 times. Most normal adults give a strong positive reaction, whereas infants and children fail to react at all. The latter finding corresponds to our results with the sera of chicks less than 1 month old, and merely reflects the slow development of antibody in the young.

TABLE 1

INCIDENCE OF *Proteus agglutinin* IN NORMAL HUMAN SERA

Group	Positive agglutination	No agglutination
Infants to 1 yr	1	13
Children 1–5 yrs	7	3
Donors (normal)	41	2
Pregnant women	14	1

Table 1 lists various normal groups and for each group the number of sera tested that agglutinated with the *Proteus* antigen contrasted with the number that did not. In infants under 1 year 1 out of 14 agglutinated, whereas in children from 1 to 5 years, 3 of 10 gave a positive reaction. It is of particular interest that in the adult groups 95% were found to have agglutinins to *Proteus,* and this includes 14 of the 15 pregnant women tested.

To continue our study we obtained sera from the Clinics and Tumor Registry of the Grace–New Haven Community Hospital and had them tested for *Proteus* agglutination by a technician. When the clinical diagnoses were received 3–4 months later the results were compared with the findings of the clinicians and are summarized in Table 2 according to a diagnosis of malignancy[3] or nonmalignancy, omitting 6 cases where the final diagnosis was questionable.

From Table 2 it may be noted that the sera from patients with various nonmalignant diseases gave an

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[3] Established by biopsy.

80% agglutination with *Proteus* antigen, whereas patients with a neoplastic growth, reported to us as untreated for their malignancy, showed an incidence of agglutination of only 28%. Statistically our value for t is 5.7, and the probability that this is a chance difference is less than 0.001.

TABLE 2

INCIDENCE OF *Proteus agglutinin* IN PATIENT'S SERA

General group	No. cases	Cases agglutinated	Agglutinated (%)	Cases not agglutinated	Not agglutinated (%)
I. Noncancer	163	130	80	33	20
II. History of cancer (treated)	24	20	83	4	17
1 No evidence recurrence	14	12	86	2	14
2 Recurrence	10	8	80	2	20
III. Active cancer (no treatment)	28	8	28	20	72

Group II has a history of cancer and has undergone such treatment as radical surgery, x-ray, and Coley's vaccine. Some show no evidence of recurrence after as long a period as 18 years, and others have extensive metastases so destructive that 2 have since succumbed to their disease. As a group, however, the treated cancer cases show an 86% agglutination with *Proteus* and present a picture similar to the noncancer patients.

With a view to reducing our percentage of error, we grouped, according to diagnosis, all noncancer cases that failed to show agglutination with *Proteus*. We have totaled and listed each group, tabulating them against a similar grouping of noncancer agglutinators (our false negatives against our correct positives).

TABLE 3

INCIDENCE OF *Proteus agglutinin*-SERA OF VARIOUS GROUPS WITH NO APPARENT MALIGNANCY

Diagnosis	Cases not agglutinated	Cases agglutinated	Cases not agglutinated (%)
Cervical erosion	4	15	21
T.B.	3	6	33
Benign growths	4	11	26
Psychiatric patients with no physical disease	5	16	23
Patients with no apparent disease	4	23	14
Miscellaneous	13	59	18

From Table 3 one can see that no particular disease has weighted the over-all error, nor can any pattern of false-negative reaction be established from the facts presented. When the chi-square method was applied to these differences in percentage not agglutinated among the several diagnoses, the variations were shown to be insignificant statistically, with $P > 0.82$.

For some time now, the *Proteus* OX19 vaccine has been used diagnostically for typhus in the Weil-Felix test, which was designed to give a positive reaction with the sera of patients having a high titer of agglutinin. The so-called febrile antigen used in this reaction is not particularly sensitive, and dilutions of serum of 1:25, 1:50, or higher are required, as well as a limited testing time of only a few minutes (*2*).

More recently, Barnes (*3*) has used *Proteus* with a slide technique and reported that 73.9% of pregnant and 41.5% of nonpregnant women agglutinated the commercial antigen *Proteus* OX19. Our technique showed agglutination in a very high percentage of all normal adults, as indicated in Table 1, but these findings may be attributed to the time element involved, as well as to our particular antigen.

To contrast our preparation with the commercial product we titrated both against immune rabbit sera. For this purpose, rabbits were immunized 2 months before the test with intravenous injections of heat-killed *Proteus* organisms. We could then determine that the commercial antigen was agglutinated by immune sera that were in a dilution of only 1:10, whereas a dilution of 1:100 or more was sufficient to agglutinate our antigen.

Our findings, which indicate a lessened incidence of *Proteus agglutinin* in patients with an untreated malignancy, find support from several authors (*4–7*), who report a paucity of antibacterial antibody, as well as an associated impairment of the immunological response in both Hodgkin's disease and leukemia. Included in these studies of antibody and immunity were *Br. abortus, S. typhosa, S. paratyphi, S. schottmülleri, T. pallidum, B. tuberculosis,* and *Proteus* OX19.

It was of further interest to find a report (*8*) of two cases, one with lymphoid leukemia, where an intravenous administration of horse serum failed to raise the heterophile antibody titer, and the other with a probable leukosarcoma where the horse serum effected a minimal increase of sheep cell agglutinins.

Although our study has indicated an infrequent occurrence of *Proteus agglutinin* among untreated cancer cases, patients who have received therapy for their malignancy do not demonstrate a similar decline. This finding would seem to suggest some restoration of the immunological mechanism.

Since we have recovered *Proteus agglutinin* in the globulin fraction, the described variation in antibody titer may reflect a change in serum globulin under neoplastic conditions.

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