

## IMMUNOLOGICAL PROPERTIES OF NATIVE AND MODIFIED DESOXYRIBONUCLEOPROTEINS OF HUMAN TISSUES

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There are a number of references in the literature to the fact that modified protein does not form antibodies to the unmodified protein and that physicochemical action sharply reduces serological activity of a number of proteins [1 - 9]. However, we found no data on immunological activity of desoxyribonucleoproteins in relation to their physicochemical state or references to the degree of modification of tissues used for immunization of animals in the study of DNP. \* Yet the study of DNP of organs and tissues must be very carefully conducted in view of the great lability of this protein.

In 1956 V.S. Gostev and D.G. Grigoryan developed a new method for serological study of DNP in which their fundamental property of solubility in 1 M NaCl solution and insolubility in physiological NaCl solution was utilized. Cotton fabric saturated with DNP solution in 1 M NaCl solution stably fixes a definite amount of protein when it is transferred to physiological NaCl solution.

This adsorbent, saturated with DNP solution, was used as test antigen in the serological reaction. Upon combining it with antisera, an increase in nitrogen was observed, which was determined according to Conway. The new method permits study of the immunological activity of DNP, which is dependent not only on the species, group and organ origin, but also on the physicochemical state of the DNP.

In the present study, experiments were conducted on sera obtained by immunizing animals with "autopsy" and with operative tissues from stomach cancer and normal stomach of a human cadaver.

DNP isolated from a stomach cancer and from human thyroid gland served as test antigen in the reaction. In both cases the material was freshly frozen operative material.

A serological study was also made of DNP fixed on cotton adsorbents and dried in air at room temperature.

The study of DNP was carried out by the method of specific fixation of nitrogen on the cotton adsorbent.

### Preparation of Antisera

Antisera were obtained by intraabdominal immunization of rabbits. Injections of increasing doses of protein were given every 24 hours for 8 days.

Animals were divided into 3 groups. The first group of rabbits was immunized with a brei of cancer tissue from human stomach, which immediately after operation was frozen with dry ice. In the future we will designate this tissue "operative tissue".

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\*DNP - desoxyribonucleoproteins.

The second group of rabbits was immunized with the same cancer tissue from human stomach, but this part of the tumor was frozen 24 hours after operation, having been kept at room temperature during these 24 hours. This tissue we will designate as "autopsy tissue".

The third group of rabbits was immunized with homogenate of normal stomach from a human cadaver (20-24 hours after death).

TABLE 1

Amount of Protein in Milligrams on Cotton Adsorbents Containing Native DNP from Stomach Cancer Tissue and from Human Thyroid Incubated with Serums against "Autopsy" and Operative Human Tissues

Experiment No.	DNP antigen from	A + DNP	A+DNP+ rabbit serum	A+DNP+ serum vs. stomach cancer "autopsy" tissue	A+DNP+ serum vs. normal stomach	A+DNP+ serum vs. stomach cancer "operative" tissue	% protein increase
85	Cancer tissue from human stomach	0.157	0.580	0.560 0.612	—	0.770	37 26
91		0.297	0.595	0.542	—	0.700 0.717	29 32
92		0.210	0.962	0.980	—	1.295	32
93		0.262	1.225	1.312 1.242	—	1.802	30 46
93a		0.262	1.225	1.312 1.242	—	1.732	32 40
94		0.350	0.682	0.700	—	1.032 1.260	47 80
69		0.140	0.542	—	0.542	0.717	32
71a		0.087	0.271	—	0.262	0.385	47
72		0.073	0.233	—	0.233	0.467	100
68	Human thyroid gland	0.153	0.840	—	0.790	—	—
70		0.052	0.192	—	0.183	0.245	34
71		0.087	0.245	—	0.262	0.385	47

•A - cotton adsorbent

•Percent protein increase in samples A + DNP + serum vs. operative tissue was calculated with respect to A + DNP + serum vs. "autopsy" tissue

From Table 1 it follows that when cotton fabric saturated with DNP is incubated with normal or immune rabbit serum the amount of protein on the adsorbents increased through the fixation of both specific and non-specific serum proteins.

The amount of protein in A + DNP with serums obtained against operative tissues differed considerably (by 26-100%) from the amount of protein obtained upon incubation of DNP with serum against "autopsy" tissue. DNP upon combination with both nonimmune rabbit serum and with serum against "autopsy" tissue from human stomach cancer fix the same amount of protein. Consequently the serum against "autopsy" tissue from stomach cancer does not contain antibodies to DNP isolated from human stomach cancer. If this were so it would give the same nitrogen increase as serum against the same tissue (except that it is operative tissue).

Analogous data were obtained with DNP isolated from human thyroid gland removed because of thyrotoxicosis. Incubation of DNP from human thyroid gland with nonimmune serum and with serum against "autopsy" tissue from a normal human cadaver gives the same results. Analogous behavior is noted in other experiments.

The next step in this work was study of serological activity of DNP of cancer tissue from human stomach adsorbed on cotton fabric and dried in air at room temperature.

Experiments were conducted similarly to the preceding ones, but with the difference that both native DNP and that modified by drying were used as test antigens (Table 2).

TABLE 2

No. of Milligrams of Protein in A+ Modified DNP of Cancer Tissue from Human Stomach upon Combination with Serums vs. "Autopsy" and Operative Tissues

Experiment No.	A+native DNP	A+modified DNP	A+DNP+normal rabbit serum	A+DNP+serum vs. "autopsy" tissue from human stomach cancer	A+DNP+serum vs. operative tissue from human stomach cancer
95	—	0.245	0.297	0.280	0.300
				0.262	0.245
					0.227
96	0.098	0.240	—	0.280	0.262
				0.227	0.542
				0.315	0.297
97	0.245	0.262	0.402	0.332	0.577
				0.420	

As seen from Table 2, DNP dried in air becomes serologically almost inactive. The nonspecific protein fixation, which occurred in the modified DNP with nonimmune rabbit serum, is observed also with serum against operative tissue from human stomach cancer.

Native DNP reacts in a completely different way with the same serums, while the amount of protein fixed in samples A + native DNP + serum against operative tissue from human stomach cancer was 0.542 mg, in samples A + native DNP + normal rabbit serum it was 0.227 mg. Analogous results were obtained in other experiments.

From the present study as well as literature data it follows that in study of serological properties of nucleoproteins the native state of the antigens used both for immunization and as test antigens is of critical significance. It must be assumed that the low serological activity of nucleoproteins of human and animal organs and tissues noted by many workers is due to the fact that antibodies to nucleoproteins were obtained by immunization of animals with cadaver tissue and that nucleoproteins isolated in most cases by extraction with alkali were used as test antigens.

Retention of immunological properties by DNP depends to a considerably greater extent on their native state than is the case with other proteins. Just as depolymerization and enzymic decomposition of nucleoproteins occur during autolysis, so, under conditions of partial drying of DNP modification occurs and in both cases the serological activity of DNP disappears.

Thus, a difference was found in formation of antibodies to DNP upon immunization of animals with freshly frozen operative tissues and with human "autopsy" tissues subjected to autolysis for 24 hours. The DNP isolated from cancer tissue from human stomach by the method of Mirsky and Pollister when combined with serums obtained by immunization with operative tissues are serologically active. Serum obtained by immunization of animals with "autopsy" tissues does not contain antibodies to DNP.

DNP dried in air at room temperature loses serological activity.

The physicochemical state of DNP is of fundamental significance to its immunological properties.

## SUMMARY

The difference in formation of antibodies to desoxynucleoproteins in immunization of animals by freshly frozen (operative) human tissues and by "autopsy" tissues, subjected to autolysis, was demonstrated by the method of specific fixation of nitrogen on cotton adsorbent. Dried at room temperature, DNP loses its serological activity. Physico-chemical condition of DNP is of considerable significance for its immunological properties.

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