A METHOD FOR THE SEROLOGICAL INVESTIGATION OF DESOXYRIBONUCLEOPROTEINS*

V. S. Gostev and D. G. Grigorian

From the Laboratory of Immunochemistry (Head – Prof. V. S. Gostev) of the Institute of Experimental Biology (Director – Prof. I. N. Maiskii Acad. Med. Sci. USSR, Moscow

(Received June 8, 1957. Presented by Active Member Acad. Med. Sci. USSR N. N. Zhukov-Verezhnikov)

Desoxyribonucleoproteins are an important component of living cells and tissues and play an essential role in many vital processes. The participation of nucleoproteins is especially great in processes of cell division and synthesis of albumins. Because malignant tumors have intense protein synthesis, the study of nucleoproteins in association with the cancer problem assumes especially great significance.

It is well known that various proteins can be studied within the organs and tissues of man and animals by numerous methods such as complement fixation, reaction of agglutination, precipitation, etc. In the investigation of desoxyribonucleoproteins, however, there is a difficulty associated with the fact that such substances are insoluble in physiological saline solution.

The desoxyribonucleoproteins dissolve either in an extremely low ionic concentration or a very high concentration, i.e., either in distilled water or in NaCl solution of 1-2 M strength. In physiological saline solution, the desoxyribonucleoproteins (DNP) precipitate as fine threads.

It is for this reason that we undertook the task of developing a method for the serological study of DNP [1], from the considerations just given.

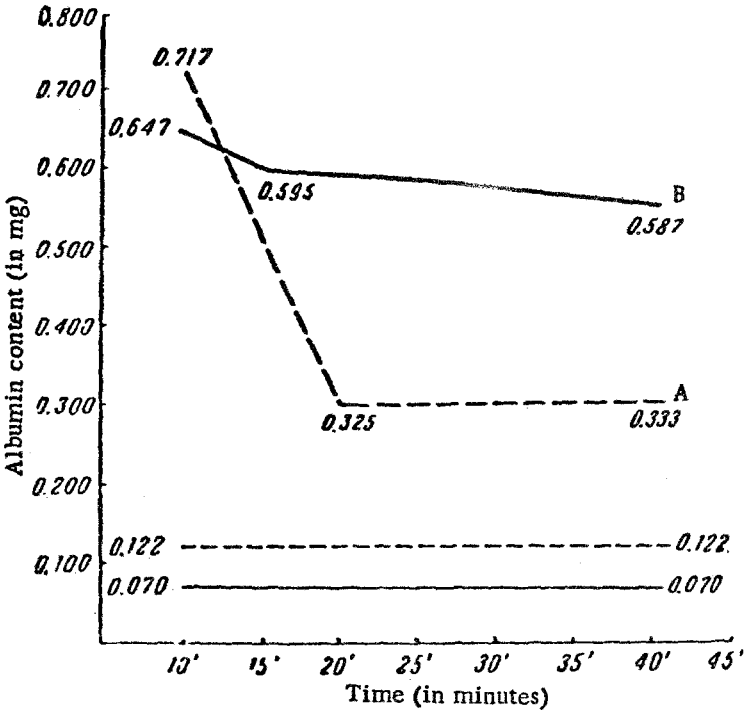
Any tissue that can be thoroughly saturated with DNP dissolved in 1 M solution of NaCl will be termed "adsorbent." Such adsorbents can be transferred into a solution of physiological saline and the DNP fixed firmly to its surface. As the adsorbent we employed "Whatmann 1" papers, filter paper, and also ordinary cotton cloth. Such an adsorbent, saturated with DNP, served us as the test antigen in the immunological reaction.

The results of the immunological interaction between the adsorbed nucleoprotein and the antiserum were judged by the increase in the amount of nitrogen which occurred as a consequence of the protein binding by the antiserum. This is the first time that such an immunological procedure is described in the literature.

EXPERIMENTAL METHODS

Preparation of test antigen DNP. Nuclear DNP was obtained for our experiments by separation from cancer stomach tissues taken from human stomachs removed by surgery. Immediately after the operation, the cancer tissue was frozen, dry ice being used in many instances. The tissues were finely sliced and washed with cold physiological saline in order to remove blood. Then the tissues were homogenized for 7-10 minutes in cold physiological saline to which an inhibitor was added at the first extraction, this agent being a 0.01 M solution of sodium desoxyribonuclease citrate. Further treatment followed the method of A Mirsky and A. Pollister [2].

* This work was presented December 11, 1956 at the Scientific Conference devoted to methods of utilizing the immunological approaches to cancer problems, the gathering being held under the auspices of the Institute of Experimental Biology of the Academy of Medical Sciences of the USSR. See the program of the conference.



Rinsing of loosely held albumins of sera with DNP adsorbed upon cotton tissues (in mg of albumin determined from the nitrogen).
A) Adsorbent + DNP – normal rabbit serum; B) adsorbent + DNP + human antigastric cancer serum.

The tissue remaining after the separation of the cytoplasmic proteins was subjected to prolonged extraction with 1 M NaCl, the process being continued for 2-3 days until a viscid solution was obtained. The undissolved tissue was separated by centrifuging.

The DNP thus obtained was purified by repeated reprecipitation the method used being dilution of the solution from 3 to 8 times by the use of six volumes of water. All the work done on separating the DNP was conducted at temperatures between 2 and 4°.

The DNP preparations so made were mineralized with sulfuric acid and the nitrogen was determined by Conway's method, while total phosphorus was determined by the method of Fiske-Subbarow. The ratio of nitrogen to the total phosphorus varied from 3.6 to 4.3 depending on the number of purifications.

Definite weighed amounts of adsorbent paper or cotton cloth, measured in quantities of 15-60 mg on torsion balances, were immersed for 20 minutes in the cooled solution of DNP dissolved in 1 M NaCl. Special experiments had taken into account the time factor (from 20 minutes to 21 hours) and had shown that DNP adsorption upon the materials was complete within the 20 minutes. In order to fix the DNP firmly upon the adsorbents and then the remove DNP material loosely precipitated, the adsorbing tissues were then transferred for 20-25 minutes into physiological saline. As a rule, the DNP became adsorbed upon the tissues very firmly. The amount of DNP fixed upon the tissues was a function of the surfaces of the adsorbents being used and their concentrations within the solution. The extent of DNP adsorption was determined using the nitrogen method of Conway. The basic potion of our experiments was done with test antigens containing from 0.052 up to 0.122 mg of protein as determined by the nitrogen content.

Preparation of Antisera. We obtained antisera by means of intraabdominal immunization of some of our rabbits by weighed quantities of malignant cancer tissues taken from surgically removed specimens of human stomachs. The remaining rabbits were immunized by homogenates taken at autopsy from normal human stomachs. The injections were done every 3 to 4 days for a total of 24 days, increasing amounts of protein (determined from the nitrogen) being used. The rabbits were exsanguinated 8 days following the last immunization.

The arrangement of the serological reaction specifically fixing the nitrogen. On hollowed glass slides there would be placed the adsorbents impregnated with the DNP. To this there would be added the undiluted immune sera in amounts of 0.2 to 0.4 cc depending upon the weight of the adsorbent. The adsorbents, saturated with the DNP, were incubated with the sera in a moist chamber at room temperature for 1 hour. During the incubation period, the specimens were turned 3-4 times. Then the specimens were placed for 25-30 minutes in physiological saline solution at room temperature. After washing, the specimens were transferred to Kjeldahl flasks for incineration. As a catalyzing agent, hydrogen peroxide was used.

EXPERIMENTAL RESULTS

The figure presents data obtained as a result of rinsing off the lightly held proteins from the sera with DNP adsorbed upon the cotton tissues.

These data indicate that the tissues adsorb and firmly hold a definite amount of desoxyribonucleoprotein which cannot be removed by rinsing. The amount of protein held by adsorption was practically the same after a 10-minute rinsing as it was after 48 hours of rinsing.

Additional Increment of Protein (expressed in mg according to the nitrogen) On Paper Antigen Saturated with DNP Taken from Human Gastric Cancer Incubated with Both Normal and Anticancer Sera

No. of experiment	Adsorbent A	A plus DNP	A plus DNP plus normal rabbit serum	A plus DNP plus anti-normal stomach serum	A plus DNP plus anti-gastric cancer serum
60a	0.017	0.105	0.385	—	0.560
60b	—	0.105	0.385	—	0.770
63	0.020	0.122	0.192	—	0.280
64	—	0.087	0.262	—	0.437
67	0.020	0.122	—	0.840	1.102
69	—	0.140	0.542	0.542	0.717
70	0.012	0.052	0.170	0.227	0.350
71	—	0.087	0.271	0.262	0.385
72a	—	0.073	0.233	0.233	0.467
72b	—	0.073	0.233	0.233	0.467

After the tissue saturated with nucleoproteins was incubated with normal or immune sera, the quantity of protein in the adsorbents increased markedly as a result of fixation of both specific and nonspecific serum proteins. However, as was to be expected, rinsing demonstrated that specific fixation of proteins by antibodies was a far firmer bond than nonspecific association of proteins with normal sera.

The quantity of protein in samples labeled "adsorbent plus DNA plus normal rabbit serum" after a 10 minute rinsing with physiological saline was 0.717 mg; after 20 minutes there was 0.325 mg of protein after further rinsing, no more protein could be removed from the adsorbent. A 20-minute rinsing removed 56% of the protein in the indicated samples.

When samples labeled "adsorbent plus DNP," (incubated with anticancer serum) were tested, it was determined that the 15-minute rinsing diminished the albumin content only by 8% (from 0.647 to 0.595) and that further rinsing removed no more albumin from the adsorbent. This must signify that the nucleoprotein adsorbed on the tissues after incubation with the corresponding antiserum, becomes quite firmly attached to the protein of the antiserum, which must then have a specific character.

In order to have comparable conditions, the specimens labeled "adsorbent plus DNP," after incubation with the normal as well as with the specific antiserum, were all rinsed for 25-30 minutes. When the specimens were rinsed after incubation with cold physiological saline solution, the amount of protein fixed was twice that

obtained by rinsing with physiological saline at room temperature. These experimental relationships were maintained rigorously.

The table presents the data of some of the experiments which demonstrate the amount of nitrogen increase in tissue paper and cotton cloth adsorbents saturated with DNP taken from human gastric cancers after being incubated with normal rabbit sera, with rabbit anti-normal stomach sera and with antisera prepared against human gastric cancers.

This table demonstrates that when adsorbed DNP taken from human cancers is incubated with anticancer sera, a definite specific fixation of proteins takes place; this phenomenon is not observed when the same DNP is incubated with normal sera or with antisera prepared against normal human stomachs. The last finding may be explained by the fact that we were forced to use autopsy material from which to prepare tissues of normal human stomachs with which rabbits were immunized.

It is well known that autopsy material will not yield native DNP because of its destruction. The DNP we extracted from the autopsy material apparently failed to cause the formation of specific antibodies as was the case against the high-polymer native DNP obtained from the surgical specimens.

Thus, when DNP loaded upon adsorbents along with the corresponding antisera is incubated, there is a surface, very firm fixation of the proteins of the specific sera which may be measured quantitatively by the nitrogen which has been fixed. This quantitative method of studying the DNP adsorbed upon cotton tissues permits an investigation of the immunologic specificities of DNP having varying origins. The method is quite simple and lends itself to ready repetition.

SUMMARY

A new method for the quantitative determination of desoxyribonucleoprotein adsorbed upon cotton materials and treated by various immunological procedures is presented.

This method has been used to demonstrate that human gastric cancers produce antibodies which can be fixed by the appropriate specific immune serum.

The procedure is described in some detail. The inherent simplicity of the procedure is emphasized.

LITERATURE CITED

[1] V. S. Gostev, D. G. Grigorian, N. A. Shagunova and N. M. Teplova, Annotations of the Scientific Studies of the Acad. Med. Sci. USSR for 1955, No. 1, 239-241.

[2] A. Mirsky and A. J. Pollister, J. Gen. Physiol., 1946, Vol. 30, pp. 117-143.