

## CONDITIONED REFLEX REGULATION OF THE SERUM GLOBULIN LEVEL

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Although it has generally been found [5] that the humoral factors of artificial immunity are under conditioned-reflex regulation, there is no unanimity on this point. No reports of special investigations of the physiological mechanisms of the conditioned-reflex regulation of the antibody level are to be found in the literature.

The present investigation was conducted on oxen used for serum production, and taking part in parallel experiments to determine the conditioned-reflex maintenance of the specific biological activity of the serum of immunized animals in industrial conditions at the Tabakhmel'skii biological products factory during the period from 1958 to 1960.

## EXPERIMENTAL METHOD

Experiments were carried out on 22 oxen used for producing a trivalent *Pasteurella* antiserum. The unconditioned stimuli used were 48-h living broth cultures of *Pasteurella bovis*, *P. ovis*, and *P. suis*, precipitated by alum. The conditioned stimulus was a group of contact and distant stimuli accompanying the stereotype of injections of antigenic stimulus. The technique of the experiments is described more fully in the paper by Dzhinukhadze et al. [3]. The technique of the biochemical investigations was as follows. Blood was taken from the jugular vein of the oxen. The serum was obtained and the following determined: 1) the total nitrogen by the Kjeldahl method, and 2) the nonprotein nitrogen by the method of protein precipitation with trichloroacetic acid followed by determination of the nitrogen in the filtrate by the Kjeldahl method. The difference between the total and nonprotein nitrogen values gave the protein nitrogen, and by multiplying this figure by 6.25, the serum protein level was deduced. In many of the investigations, however, the total protein was measured directly by means of a nephelometer.

The serum proteins, and in particular the globulin fraction, were studied by paper electrophoresis on filter paper in a refrigerator [2] for 18-20 h in a chamber of the type used by Flynn and de Mayo [6], with a medinal-veronal buffer at pH 8.6 and ionic strength 0.1, the voltage between the poles of the electrodes being 220-240 V and using M-3 chromatographic paper. The electrophoregrams were read quantitatively by means of a photoelectric cell [1]. Since the  $\beta$ - and  $\gamma$ -globulins in the ox serum were not clearly distinguishable, to avoid error they were determined together.

The specificity of the globulins produced under the influence of the conditioned-reflex stimuli was studied by biological tests of the immunological activity of the serum of the producer oxen on rabbits and albino mice.

## EXPERIMENTAL RESULTS

Preliminary experiments showed that the total nitrogen in the serum of the oxen during hyperimmunization rose significantly, whereas the nonprotein nitrogen concentration remained practically unchanged. The total protein accordingly increased, and this was due mainly to an increase in the globulin fraction of the protein, the serum globulin level remaining relatively unchanged.

In the first series of experiments 10 oxen were used (5 experimental animals and 5 controls—Nos. 2, 5, 8, 9, and 10). Seven months after the beginning of the hyperimmunization cycle, systematic injections of the *Pasteurella* antigen were discontinued, and starting on March 17, 1954, while the conditions of the experimental stereotype were otherwise strictly observed, instead of antigen the oxen of the experimental and control groups received injections of physiological saline or sterile broth with alum. Subsequently the oxen of the experimental group received booster injections, each of 50 ml of antigen, once per annum. In the intervals between injections of antigen, the immunological activity of the serum of the oxen was maintained by a conditioned-reflex method—by injection

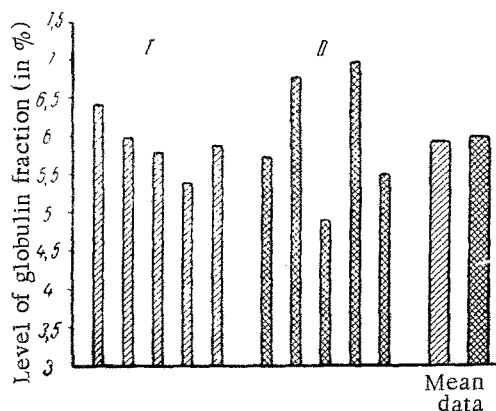


Fig. 1. Conditioned-reflex maintenance of a high level of the globulin fraction of the serum proteins in experimental (I) and control (II) animals 4 months after the last injection of antigen. Along the axis of abscissas—individual animals.

and 39). The results again demonstrated that a conditioned-reflex mechanism was responsible for maintaining the high level of the globulin fraction of the protein. Calculation of the mean values of the relative percentage of the globulin fraction of the serum protein showed that in the control animals, receiving systematic injections of the antigenic stimulus, it was only 0.02% greater than in the oxen exposed only to the action of the series of conditioned stimuli.

Comparison of the results of the first and second series of experiments, conducted on the same oxen (Nos. 2, 5, 8, 9, and 10), shows that this series of conditioned stimuli led to the prolonged (for 4-6 months) maintenance of the globulin fraction of the protein at a high level, whereas in the control oxen a high percentage of the globulin fraction was found only for 1.5-2 months after the cessation of the injections of antigen.

The third series of experiments was carried out on 5 oxen not previously used in the investigation (Nos. 1 and 4—experimental, and Nos. 2, 5, and 6—control) in order to examine the after-effect of antigenic stimulation, maintaining the antibody-forming activity of the immunogenic apparatus at a high level. From May 4 until June 9, 1958 a cycle of hyperimmunization was undertaken in all 5 animals, using the normal routine of the Tabakhmel'skii factory [initially 4 injections of a heated culture of the microorganisms (*Pasteurella*), followed by 8 injections of a living culture], after which oxen Nos. 1 and 4 received injections of broth alone, the other experimental conditions being the same, while oxen Nos. 5 and 6 received further injections of antigen, but at intervals of 12-13 days, and ox No. 2 received no further injections of either antigen or broth, so that the conditioned-reflex mechanism of maintenance of the high immunological activity of the serum was not in operation. Subsequently (from July 2 to August 7, 1958) we investigated the relative percentage of the globulin fraction of the protein in all the animals separately. The changes in this level in the experimental (Nos. 1 and 4) and control (Nos. 5 and 6) oxen are shown in Fig. 2. The globulin level in the experimental and control animals was very little different, thus confirming the results of the preceding series of experiments. The most interesting feature of this series of experiments was the results of the investigation of the globulin level in control ox No. 2 (Fig. 3). This experiment showed that 2 months after discontinuing the antigen injections and in the absence of conditioned stimulation the level of the globulin fraction fell sharply—from 6.06 to 4.36%, i. e., approximately to the control level (3.5-4.5%). The results of the study of the rate of fall of the high content of the globulin fraction of the protein (2 months) agreed with those obtained by other workers [3].

To conclude the investigation, we carried out a further series of control experiments in order to discover the effect of the foremost component (intravenous injections of broth) of the situational conditioned stimulus on the relative percentage of the globulin fraction of the serum, i. e., to verify that the conditioned stimulus was indifferent in relation to the reaction under study. For this purpose two oxen were studied (without preliminary hyperimmunization); after the preliminary investigation of the initial level of the globulin fraction, the animals received

of broth. So far as the oxen of the control group (Nos. 11, 12, 13, 14, and 15) are concerned, during the period described (1954-1958) *Pasteurella* antigen continued to be injected every 12-13 days (100 ml of a virulent culture to each animal).

Four months after a routine booster injection of antigen had been given to the experimental oxen, followed by the application of conditioned stimuli, the level of the serum globulin fraction was investigated separately in all the animals. The total protein, albumins and globulins were determined simultaneously in all the oxen of the control group.

The results of these experiments showed (Fig. 1) that the relative proportion of the globulin fraction did not differ significantly in the two groups of animals, thus demonstrating convincingly that the globulins were maintained at a high level by a conditioned-reflex mechanism.

In the next series of experiments we again investigated the serum globulin level under the influence of the complex situational conditioned stimulus, but this time 6 months after the last booster injection of antigen. Experiments were conducted on 10 oxen: 5 experimental (Nos. 2, 5, 8, 9, and 10) and 5 control (Nos. 3, 6, 36, 38,

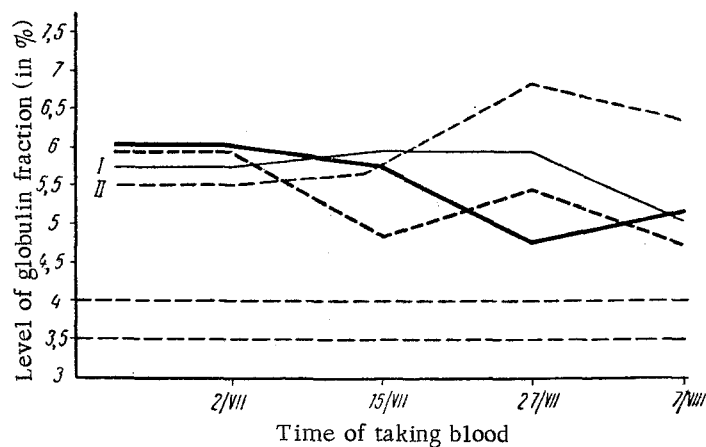


Fig. 2. Changes in the level and concentration of serum globulins in experimental (I) and control (II) animals under the influence of the conditioned stimulus and of systematic injections of antigen.

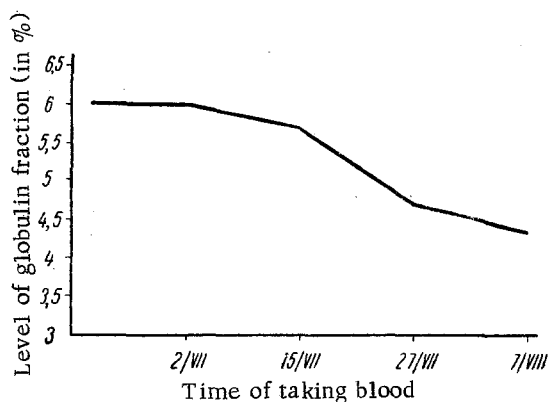


Fig. 3. Fall in the level of the serum globulins of a control animal after cessation of injections of antigen.

repeated injections of broth with alum. The broth injections caused no detectable increase in the serum globulin or slightly increased the total protein concentration as a result of a slight increase in the albumins. It followed, therefore, that the prolonged maintenance of the high level of the serum globulins observed during this investigation could be attributed to the conditioned-reflex component of the nervous regulation of the synthesis of the serum globulins. The injections of broth did not themselves stimulate globulin synthesis, in the manner of an anamnestic reaction; for this to develop it would be necessary for the second antigen to be closely similar or identical to the first [4, 7, 8].

#### SUMMARY

Experiments were conducted on oxen to study the conditioned-reflex mechanism responsible for maintaining the serum globulins at a high level. The results confirmed the theory that the immunological reactions of the organisms are

regulated by a conditioned-reflex mechanism and shed light on the physiological mechanisms of the cortical control of antibody synthesis. Apparently conditioned-reflex stimuli modify the intensity of the general nonspecific function of the immunogenetic apparatus, namely the synthesis of immune globulins, and thereby regulate the synthesis and production of specific antibodies.

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