Thus MESI, when injected parenterally in certain doses for a certain period of time has an antiatherosclerotic action. It may also be postulated that in all probability MESI has no direct anticoagulant action, but when the changes of hemostasis revealed by this investigation are interpreted, it can be tentatively suggested that multiple administration of MESI stimulates endogenous heparin production.

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# IMMUNIZING ALBINO RATS WITH A COVALENT SYDNOPHEN–SERUM ALBUMIN CONJUGATE DEPRESSES CHRONIC ETHANOL CONSUMPTION


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UDC 612.017.1+613.816+591.51

KEY WORDS: sydnophen; conjugates; immunization; alcoholism; rats.


The use of immunologic methods inducing long-term changes in physiological status offers fundamentally new opportunities. Essentials of methods of this type are examined in a review [1] and can be summarized as immunization of an animal with covalent conjugates of carrier antigens with biological regulators, identical or similar to the intrinsic regulatory compounds of the animal itself; the formation of antibodies binding the corresponding endogenous regulator (or group of regulators) long enough for intensive antibody production to take place (usually months) is induced, and changes take place in the level of the regulator, the general balance of the regulatory systems, and, ultimately, several physiological functions.

The long-term effect, resembling in many of its features the action of neuroleptics [4], was described previously during immunization of rats with a covalent conjugate of sydnophen (an antidepressant and neurostimulator, which interferes with the action of catecholamines) with bovine serum albumin (BSA). The possibility of immunologic depression of alcohol motivation has been demonstrated in several publications [3, 5, 6]. The aim of this investigation was to study the effect of immunization with a conjugate of sydnophen on the attitude of albino rats to chronic ethanol consumption.

## EXPERIMENTAL METHOD

Sydnophen was conjugated with BSA with the aid of glutaraldehyde [4]. By varying the molar proportions of the components, conjugates containing from 5 to 33 moles of sydnophen to 1 mole of protein were obtained. The experiments were conducted on 82 noninbred male albino rats weighing 150-200 g. Immunization consisted of three injections of conjugate


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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 12, pp. 695-697, December, 1989. Original article submitted February 2, 1989.

TABLE 1. Mean Daily Ethanol Consumption (mean values for group; in g/kg) at Different Times after Immunization with Conjugate of Sydnophen with BSA

| Conjugates containing sydnophen, moles/mole protein | Period after immunization, days | | | |
|---|---|---|---|---|
| | 20—40 | 40—60 | 60—80 | 80—100 |
| 5 ($n = 7$) | 0,79 | 2,6 | 1,9 | 3,5 |
| 18,6 ($n = 9$) | 0,46* | 0,2** | 0,19 | 0,73** |
| 19,2 ($n = 10$) | 0,65** | 0,3** | 1,76** | 1,97 |
| 33 ($n = 5$) | 0,13** | 0,15** | 0,33** | 0,3** |
| Control (n = 30) | 1,2 | 2,66 | 2,56 | 2,73 |
| BSA (n = 22) | 0,51 | 1,34 | 2,4 | 1,87 |

Legend. n) Number of animals in group; statistically significant differences: *p < 0.05, **p < 0.01.

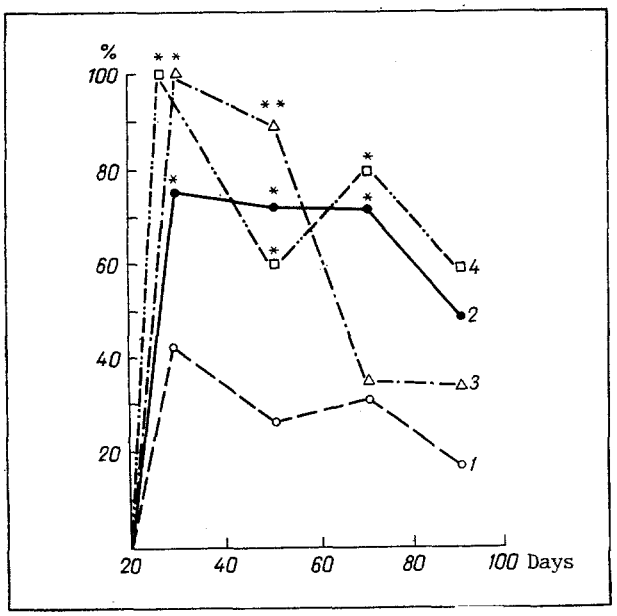


Fig. 1. Number of animals consuming ethanol at the rate of 0-1 g/kg body weight at different times after immunization with conjugates of sydnophen and BSA. Abscissa, time after immunization (in days); ordinate, number of animals (in %). 1) Control, 2, 3, 4) conjugates with 18.6, 19.2, and 33 moles of bound sydnophen molecules to 1 mole of protein respectively. Statistically significant differences: p* < 0.05; **p < 0.01.

subcutaneously at several points in the dorsal region at intervals of 7-8 days; for the first two injections the conjugate was mixed with Freund's complete adjuvant (FCA; 1:1). The dose expressed as protein was 150 μg per rat. The following were injected in the control experiments: 1) BSA without sydnophen, treated with glutaraldehyde, and with FCA; 2) physiological saline. Antibody titers were determined 2 months after the first immunization by ELISA, using phosphate planting buffer, pH 7.5; values were 1/64-1/2048.

Alcoholization of the control and immunized animals was carried out under free choice conditions [2]. On the 21st day after the beginning of immunization the rats were placed in individual cages measuring 25 × 25 × 20 cm, containing two bowls (with 15% ethanol solution and with water). The quantity of water and ethanol solution drunk by each rat per day was recorded. The results were subjected to statistical analysis by the Fisher and Wilcoxon—Mann—Whitney tests.

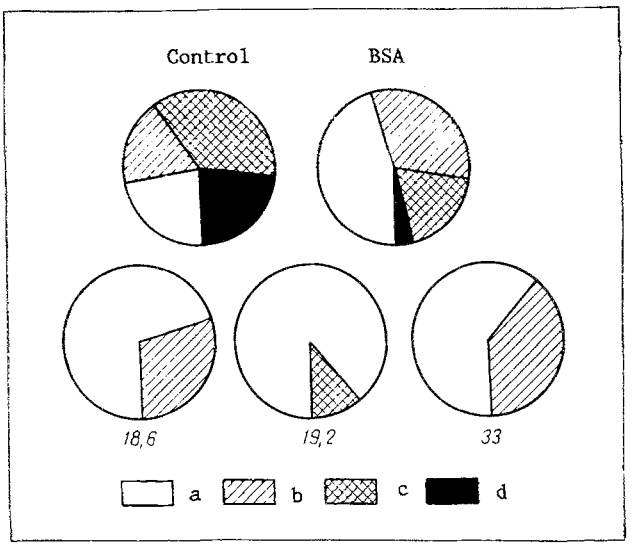


Fig. 2. Distribution of animals in control and immunized groups by quantity of ethanol consumed (between 40 and 60 days after immunization). Numbers indicate number of bound sydnophen molecules in conjugate (in moles/mole protein). Quantity of ethanol consumed (in g/kg body weight): a) 0-1, b) 1-2, c) 2-5, d) >5.

TABLE 2. Number of Animals (in %) with Moderate and Strong Addiction to Alcohol (ethanol consumption ≥ 3 g/kg body weight) at Different Times after Immunization with Conjugate of Sydnophen with BSA

| Conjugates containing sydnophen, moles/mole protein | Period after immunization, days | | | |
|---|---|---|---|---|
| | 40—60 | 60—80 | 80—100 | 100—120 |
| 5 ($n = 7$) | 0 | 0 | 14,3 | 57,1 |
| 18,6 ($n = 10$) | 0 | 28,6 | 0 | 33,3 |
| 19,2 ($n = 9$) | 0 | 12,5 | 25 | 37,5 |
| 33 ($n = 5$) | 0 | 0 | 0 | 0 |
| Control (n = 30) | 50 | 40 | 41,2 | 52,9 |
| BSA (n = 22) | 15 | 21 | 17 | 50 |

Legend. n) Number of animals in group.

## EXPERIMENTAL RESULTS

Immunization of the rats with conjugates of sydnophen with BSA (content of bound sydnophen between 18.6 and 33 moles/mole protein) caused a considerable decrease in the volume of ethanol consumed when water and 15% ethanol solution were provided (Table 1). These changes began to appear with effect from the first days of alcoholization in the rats (i.e., 3 weeks after the beginning of immunization of the animals) and they continued for 2-3 months or, in some cases, for longer after immunization. The decrease in the volume of alcohol consumed by the group as a whole (Table 1) could be attributed both to an increase in the number of animals virtually refusing alcohol (Figs. 1 and 2) and to a decrease in the number of animals with moderate or strong addiction to alcohol compared with the control group and the group immunized with BSA (Table 2; Fig. 2). During the period of 20-40 days from the beginning of immunization all the animals virtually refused alcohol (Fig. 1), but in the period up to 2 months, in all the experimental groups there was not a single rat with moderate, or still less, strong addiction to alcohol (Table 2). This effect was not connected with a general decline of fluid consumption by the immunized rats.

The conjugate with a low content of bound sydnophen (5 moles/mole protein) was the least effective in this case. In the group of animals immunized with this conjugate there was a small, but not statistically significant change in the mean daily ethanol consumption

for the group as a whole; the number of rats refusing alcohol was virtually not increased, but animals with moderate and strong addiction to alcohol likewise were absent in this group during the first 2 months of alcoholization (80 days after the beginning of immunization, see Table 2).

Immunization of the rats with BSA, not conjugated with sydnophen, caused a small, not statistically significant, decrease in ethanol consumption in the first stages of alcoholization, which is not in agreement with the observations of the authors cited above [3, 6]. This may be connected with differences in the schedule of immunization and in the dose of BSA.

These investigations also indicate that by using the method of immunization to the conjugate, experimental alcoholism which has already been formed can evidently be depressed. The effect of immunization was manifested particularly considerably in rats with strong addiction to alcohol (consuming more than 7 g/kg body weight daily). The greatest decrease (to 0.83 g/kg) was observed between the 40th and 60th days after immunization, but even 160 days after immunization the mean daily ethanol consumption of these rats did not exceed 4 g/kg. However, a quantitative assessment of the degree and dynamics of weakening of already existing addiction to alcoholism by the conjugate requires closer study in the future.

The results are thus evidence that development of addiction to alcohol can be prevented for a considerable length of time by immunization of rats with a conjugate of BSA and sydnophen, and they also show the promising nature of further research based on the principle enunciated above, with the aim of suppressing experimental alcoholism which has already been formed.

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