for long-term keeping (more than 24 h), and in the presence of high concentrations of certain biological macromolecules (in particular, lipids). However, the simplicity, ready availability, and nontoxicity of this substrate, the production of the lactamase conjugate by a very simple conjugation technique (the glutaraldehyde method), and its stability (in our experiments the lactamase conjugate did not suffer any loss of enzyme activity during keeping for 18 months at 4°C) all give the hope that conjugates based on β-lactamase will be widely used for the construction of various immunoenzyme test systems.

LITERATURE CITED

1. A. Voller, D. E. Bidwell, and A. Bartlett, Bull. WHO, 53, No. 1, 155 (1976).
2. M. Dixon and E. Webb, Enzymes [Russian translation], Vol. 3, Moscow (1982), p. 906.
3. M. I. Levi, T. A. Shimko, A. Yu. Sazykin, et al., Antibiotiki, 30, 662 (1985).
4. M. V. Sokolova, N. G. Yaroslavtseva, M. L. Khristova, et al., Vopr. Virusol., No. 6, 668 (1983).
5. I. G. Kharitonenkov, M. L. Khristova, and É. K. Ruuge, Mol. Biol., 11, 432 (1977).
6. S. M. Chaikovskaya and T. G. Venkina, Antibiotiki, 7, 453 (1962).
7. S. I. Chernykh, L. N. Stel'mashenko, T. V. Sorochinskaya, et al., Abstracts of Proceedings of the 16th Conference of the Federation of European Biochemical Societies [in Russian], Moscow (1984), p. 380.
8. R. P. Ambler and G. K. Scott, Proc. Natl. Acad. Sci. USA, 75, 3732 (1978).
9. J. S. Emtage, W. C. A. Tacon, G. H. Catlin, et al., Nature, 283, 171 (1980).
10. E. Ishikawa, M. Imagawa, S. Hashida, et al., J. Immunoassay, 4, 209 (1983).
11. G. W. Jack and M. H. Richmond, J. Gen. Microbiol., 61, 43 (1970).
12. P. K. Nakane and A. Kawaoi, J. Histochem. Cytochem., 22, 1084 (1974).
13. S. Ohya, Y. Fujii-Kuroyama, M. Yamamoto, et al., Microbiol. Immunol., 24, 815 (1980).
14. P. R. Prasal, I. Khart, and B. C. Harinath, IRCS Med. Sci. Biochem., 12, 738 (1984).
15. R. H. Yolken, S. B. Wee, and M. V. Regenmortel, J. Immunol. Meth., 73, 109 (1984).

METHOD OF IDENTIFICATION AND ISOLATION OF ORGANS OF ENDOCRINE SECRETION IN MICE

N. K. Kashirina

UDC 616.43/.45-092.9-091:57.089.6

KEY WORDS: endocrine secretion; identification

Noninbred and inbred mice have become the animals most widely used in scientific research because of their small size, rapid reproduction and development, and low cost of feeding and maintenance. A very important advantage of mice as experimental animals is the small doses of biochemical, pharmacological, toxicological, and other preparations used experimentally, which are often very expensive. In particular, we chose mice for electron-autoradiographic experiments, mainly on the grounds of the cost of labeled compounds.

Organs of endocrine secretion were removed from mice to study the difficult problems of identification and isolation of these organs. This is due to the small size of the mice themselves, their very small body weight, and some features of the arrangement of their endocrine organs. No description of such an investigation could be found in the literature, and the methods used to discover and isolate the adrenals, pituitary, and thyroid gland are described below (Table 1).

TABLE 1. Parameters of Weight of Endocrine Organs in Mice

Organ	Weight	
	mg	mg/100 g body weight
Pituitary	1,2±0,18	6,45±0,21
Adrenals	2,43±0,36	10,71±0,87
Thyroid gland	3,22±0,43	13,25±0,52

Department of Histology, Voroshilovgrad Medical Institute.

(Presented by Academician of the Academy of Sciences of the USSR, D. S. Sarkisov.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 103, No. 5, pp. 630-631, May, 1987.

0007-4888/87/0005- 0718$12.50

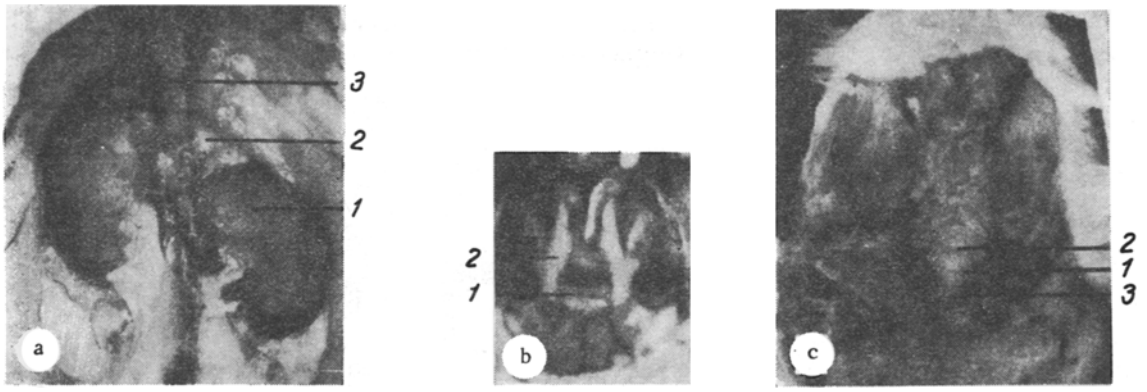

Fig. 1. Topography of endocrine organs in the albino mouse: a) retroperitoneal space: 1) kidney; 2) adrenals; 3) trunk of abdominal aorta; b) base of the skull; 1) pituitary; 2) nerve trunks (optic); 3) trachea. Magnification: 1:3.

The adrenals are closely connected topographically with the kidney. The retroperitoneal fascia splits into two layers near the lateral border of the kidney and surrounds the kidney, adrenal, ureter, and perinephric fatty areolar tissue. Near the spine the retroperitoneal fascia becomes interwoven with the membrane of the great vessles: the aorta, inferior vena cava, and renal vessels. Thus the renal receptacles are formed above and laterally by the chest wall, the lumbar muscles, transverse fascia, and the retroperitoneal cellular tissue proper, and medially by the spine and the membranes of the great vessels, and anteriorly by the diaphragm. In mice the kidneys are located at the sides of the spine, in the lateral compartment of the retroperitoneal space at the level of the 10th thoracic vertebra and the first and second lumbar vertebrae, the right kidney being 3-4 mm anteriorly to the left. During isolation of the adrenals in mice it is better to use an oblique lumber extraperitoneal approach with retraction of the wound toward the medial side and resection of the 11th and 12th ribs. After division of the posterior renal fascia the kidney is isolated. The adrenals in mice are located in the perinephric fatty tissue on the anterior pole of the kidney. They are oval or circular in shape, with a small depression on the medial surface — the hilus, where the center artery enters and the adrenal vein leaves; the lymphatic vessels of the adrenals are also located here (Fig. 1a). When the kidney is grasped to isolate the adrenals, the latter remain in the fatty areolar tissue close to the abdominal aorta, cranially and medially to the kidneys. The distance between the kidney and adrenal is increased to 8-11 mm. In the mass of the perinephric fatty tissue the adrenals can be distinguished by their dark brown color, and to isolate them is necessary to separate layers of the perinephric fatty tissue in the direction from the kidney toward the trunk of the abdominal aorta.

The pituitary gland in mice is located at the base of the skull in the region of the middle cranial fossa — in the fossa of the sella turcica of the sphenoid bone. The dura mater, which lines the fossa of the sella turcica, forms a circular fold above it, which approaches the pituitary in the form of a diaphragm, with a round hole in the middle for the pituitary stalk. Around the edges of the fossa of the sella turcica the dura is densely fused with the base of the skull by means of the Pacchionian granulations. To obtain access to the basis of the skull, the cranium must be opened, the cranial nerves divided, and the brain removed. During these manipulations the thin pituitary stalk (diameter 0.7-1.0 mm), connecting it with the hypothalamic region, is inevitably torn. The cylindrical shape of the pituitary, covered above with the dura mater, is whitish-gray in color and similar in external appearance to the nerve trunks located along the sides of the fossa of the sella turcica (the oculomotor, optic, and trochlear nerves), which also are covered with dura mater (Fig. 1b). The criterion for identification of the pituitary gland is its central position relative to the nerve trunks.

The thyroid gland consists of two lobes and a very thin isthmus (Fig. 1c). The lobes of the gland have the appearance of somewhat flattened, elongated formations, which are firmly fused with the lateral portions of the laminae of the thyroid cartilage, and descend 2-2.5 mm along the lateral surfaces of the trachea. The gland is very small in size (about 1 × 1 × 2.5-3 mm), covered with muscle fibers, and difficult to distinguish among them. When the thyroid gland is isolated, part of the larynx and the adjacent part of the trachea must be dissected, the larynx with a portion of the trachea (about 5 mm) must be excised, and the

esophagus, which is fused with the posterior wall of the trachea, must be separated. Next, having fixed the trachea with a dissecting meedle, the thyroid gland, whose capsule is firmly fused with the larynx and trachea, is due away from them.

PRODUCTION OF MONOCLONAL ANTIBODIES TO HORSERADISH PEROXIDASE AND THEIR USE IN IMMUNOHISTOCHEMISTRY AND IMMUNOBLOTTING

A. I. Faerman, A. V. Chervonskii, T. A. Chipysheva, V. I. Gel'shtein, and A. Yu. Kolyada

UDC 615.373:57.083.33].012.6

KEY WORDS: immunoenzyme tests; horseradish peroxidase; monoclonal antibodies

The unlabeled antibodies method, or peroxidase-antiperoxidase method (the PAP test) [10] is widely used in immunohistochemistry, because of its high sensitivity and the low level of nonspecific (background) staining. The introduction of monoclonal mouse and rat antibodies into immunohistochemical practice has necessitated the obtaining of large quantities of mouse or rat antibodies to horseradish peroxidase (HRP) in order to prepare reagents for the PAP test. Unlimited quantities of standard antibodies to HRP can be obtained by hybridoma technology [5, 9].

The aim of this investigation was to obtain monoclonal antibodies (MCAB) to HRP, suitable for use in different kinds of immunoenzyme assays.

To obtain hybridomas, BALB/c mice were immunized by intraperitoneal injection of HRP (type VI, from Sigma, USA); at the first immunization the antigen was injected in Freund's complete adjuvant, but during subsequent immunizations, in incomplete adjuvant (Gibco, USA). The mice were stimulated 4 days before fusion of the immune spleen cells with myeloma cells, by intravenous injection of HRP. Fusion was carried out with the aid of polyethylene-glycol 1500 (Merck, West Germany) by the method described in [1]. After hybridization the cells were transferred to 96-well plates (Linbro, England) in medium RPMI-1640. The cells were grown at 37°C in an atmosphere with 5% CO_2. The presence of antibodies to HRMP in the culture fluids (CF) was determined by solid-phase radioimmunoassay (RIA) [9]. Positive CF were tested for suitability for use in the PAP test with the aid of monoclonal antibody L9 [3, 9].

For immunohistochemical staining cryostat sections of biopsy specimens of human breast, frozen in liquid nitrogen, were fixed with 4% formalin in buffered physiological saline (BPS) for 5 min at room temperature, washed with PBS, and then incubated with CF from a hydridoma producing MCAB PK S-12 to prekeratins [4], rabbit antiserum against mouse immunoglobulins (RAM; 1:50), and monoclonal PAP reagent, obtained by addition of HRP (Reanal, Hungary), up to a final concentration of 50 μg/ml, to CF of hybridoma clone AP-FC-2B4, producing MCAB to HRP.

Electrophoresis of the microsomal fraction of rat liver cells was carried out in a polyacrylamide gel gradient (6-9%) in the presence of sodium dodecylsulfate by the method in [7]. The separated proteins were transferred to a nitroclelulose membrane [12] and stained with MCAB to cytochrome P-450 [2], RAM (1:100), and monoclonal PAP complex.

Peroxidase activity was revealed in all cases by the diaminobenzidine test [6].

After hybridoma fusion, 106 primary hybridoma cultures were tested by RIA for production of antibodies binding with HRP. Of this number, 16 were positive. However, testing by immunocytochemical staining showed that only four cultures produced antibodies suitable for obtaining an active PAP reagent. The very first testing made it clear that the most intense staining could be obtained with MCAB of the clone designated AP-FC-2B4. This result also was confirmed on testing FC from subclones for all four cultures, and accordingly MCAB of clone

Laboratory of Cell and Tissue Culture, Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR. Laboratory of Immunochemistry, Laboratory of Mechanisms of Carcinogenesis, and Laboratory of Carcinogenic Substances, Research Institute of Carcinogenesis, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR, N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 103, No. 5, pp. 631-634, May, 1987. Original article submitted June 6, 1986.

 0007-4888/87/0005- 0720$12.50