ster, R. M., Quastel, J. H., Academic Press, vol. II, 1963, 437.

12. Bengtsson, S., Philipson, L., Persson, H., Laurent, T. C., Virology, 1964, v24, 617.

13. Philipson, L., Albertsson, P. Å., Frick, G., ibid., 1960, v11, 553.

14. Holland, J. J., ibid., 1962, v16, 163.

15. Philipson, L., Bengtsson, S., ibid., 1962, v18, 457.

16. Feltz, E. T., Regelson, W., Nature, 1962, v196, 642.

17. Korn, E. D., J. Biol. Chem., 1962, v237, 3423.

18. Boeyé, A., Virology, 1963, v21, 587.

Received July 14, 1964. P.S.E.B.M., 1965, v118.

Titration of Live Measles and Smallpox Vaccines by Jet Inoculation of Susceptible Children. (29753)

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The results of a series of studies conducted in the United States and in the Republic of Upper Volta indicated that jet-inoculated live measles and smallpox vaccines immunized 97 to 100% of susceptible recipients(1). Most of these children were inoculated with a fixed dose of each attenuated virus, 10^4 tissue culture infectious $dose_{50}$ (ID_{50}) of measles virus and 10^6 chorio-allantoic membrane (CAM) infectious units of vaccinia virus, either singly or in combination.

The present study was designed to determine the effective dose range of these vaccines when given by jet injection as compared to administration by conventional techniques. In addition, an attempt was made to correlate the results of virus titrations in man with those obtained when identical samples of each of the vaccines were tested in the laboratory in conventional potency assay systems.

The clinical work was carried out in Bobo-Dioulasso, Upper Volta, with the assistance of the Upper Volta Health Ministry; laboratory tests were performed at the National Institutes of Health, Bethesda, Md.

Materials and methods. Vaccines. Lyophilized Enders "B" level(2) live attenuated measles vaccine was purchased commercially* in bottles containing fifty 0.5 ml human doses. The Division of Biologics Standards Reference Smallpox Vaccine Lot 2, a lyophilized calf lymph preparation, was used. Vaccines were refrigerated during air shipment to Upper Volta and subsequent storage until used, *i.e.*, a period of about 2 weeks, and were rehydrated with sterile distilled water immediately prior to use. Ten-fold dilutions of freshly reconstituted vaccines were made with 2 ml syringes by serial transfer of 2.0 ml volumes to rubber-stoppered vaccine bottles, each containing 18.0 ml of diluent (medium 199 containing 5% human serum albumin and antibiotics). Rehydrated and diluted vaccines were refrigerated and used within 2 hours.

Vaccinations. The 216 children, age 8 months to 6 years, participating in the study had no history of previous experience with the virus to be administered. Each vaccine dilution was inoculated into 10 or more children. The automatic jet injection apparatus† was regulated to deliver 0.5 ml of vaccine. The operating directions furnished with the apparatus were followed except for smallpox vaccination, where a short length of stiff plastic tubing was fitted over the jet gun nozzle, creating a sleeve that extended 4 mm beyond the nozzle orifice. This simple modification, described in our earlier studies(1), increased the amount of vaccine deposited intradermally. Conventional measles immunization was accomplished by subcutaneous inoculation of 0.5 ml volumes with disposable 2 ml syringes and 21-gauge, 1 inch needles. Percutaneous smallpox vaccination was done with the multiple pressure technique using

* Merck Sharp & Dohme Lot 94216/7064B

† Scientific Instruments Mfg. Corp., New York City.

30 insertions. All inoculations were made into the deltoid area after cleansing the skin with acetone. Smallpox vaccinees were requested to return periodically for 2 weeks for evaluation of skin lesions. All vaccinations were administered by one physician while another recorded subsequent cutaneous reactions.

Serologic tests. Venous blood samples were obtained from children immediately before vaccination and 21 days later. Sera were removed aseptically from clots and maintained at —20°C until air-shipped under refrigeration to the NIH. The hemagglutination-inhibition (HAI) techniques used to assay measles and smallpox antibodies have been described elsewhere(3,4). Initial serum dilutions of 1:8 and 1:10 were employed for measles and smallpox respectively. Sero-conversion was considered to have occurred if a serum specimen collected prior to inoculation contained no demonstrable antibody for the virus in question, while the sample obtained 3 weeks later had an antibody titer of at least 1:16 for measles and 1:20 for vaccinia.

Laboratory assay of vaccines. Titrations of vaccines in either cell cultures or embryonated eggs, or both, were carried out by standard techniques(5,6). Primary rabbit and monkey kidney cultures and BS-C-1 cell cultures(7) were obtained from the tissue culture section of the DBS. Inoculated stationary tube cultures were incubated at 35°C and maintained in Eagle's basal medium containing 2% inactivated calf serum and antibiotics. The medium was changed after 5 to 7 days and the endpoint readings made 12 to 14 days after inoculation. Virus titers in laboratory hosts and in man were calculated by the Karber 50% endpoint method(8).

Results. Measles vaccination. Analysis of results in the study will be restricted to 126 of the 216 children inoculated with one or the other of the vaccines. These individuals are those who proved to be devoid of antibody before inoculation and who donated a second blood specimen 21 days later. Thus, calculations of vaccine titer are limited to sero-negative children whose subsequent serologic response can be documented.

Table I summarizes results obtained when measles vaccine was titrated in susceptible children by jet injection and by conventional inoculation with syringe and needle in comparison to cell culture assay. The ID_{50} of the attenuated measles virus as indicated by sero-conversion of susceptible children was $10^{-4.2}$ (jet injection) and $10^{-3.9}$ (conventional inoculation) as compared to $10^{-4.1}$ in BS-C-1 cell cultures. These slight differences in titer are within the expected experimental variation. The data further indicated that the levels of measles antibody attained following jet injection were comparable to those observed after conventional inoculation. Also, the antibody titer ranges in children responding to minimal amounts of virus, *i.e.*, the 10^{-3} and 10^{-4} dilutions, were similar to those observed in individuals inoculated with 1000 to 10,000 times more virus.

TABLE I. Titration of Measles Vaccine in Susceptible Children and in Cell Cultures.

Inoculation	Vaccine dilution	Sero-conversion*	ID_{50}†	Antibody titer range‡
Children—				
Subcutaneous	10^{0}	10/10		64–512
	10^{-1}	7/7		32–512
	10^{-2}	8/8	3.9	32–256
	10^{-3}	5/6		64–256
	10^{-4}	2/8		128–256
Jet injection	10^{0}	2/2		128–256
	10^{-1}	3/3		128–256
	10^{-2}	7/7	4.2	64–256
	10^{-3}	5/5		64–256
	10^{-4}	2/5		32
BS-C-1 cell cultures			4.1	

* No. positive/No. tested.
† Expressed as the dilution $\log_{10}$/1.0 ml of vaccine.
‡ Reciprocal of serum dilution.

Smallpox vaccination. The results obtained with smallpox vaccine are summarized in Table II. The response of vaccinated individuals was evaluated by two methods: (1) observation of the characteristic skin lesion associated with primary vaccination, referred to here as the cutaneous ID_{50} (CID_{50}) and (2) determination of antibody response, referred to here as the immunologic ID_{50} (IID_{50}), irrespective of cutaneous response.

When smallpox vaccine was introduced into susceptible children by the percutaneous route the CID_{50} and the IID_{50} were essen-

TABLE II. Titration of Smallpox Vaccine in Susceptible Children and in Laboratory Systems.

Inoculation	Vaccine dilution	Primary cutaneous lesions*	Cutaneous ID_{50}†	Sero-conversion*	Immunologic ID_{50}†	Antibody titer range‡
Children—						
Scarification	10^{-1}	8/8		8/8		80– 320
	10^{-2}	4/6		4/6		80– 160
	10^{-3}	2/5	2.6	1/5	2.4	160
	10^{-4}	0/4		0/4		—
	10^{-5}	0/9		0/9		—
Jet injection	10^{-2}	6/6		7/7		80– 640
	10^{-3}	4/6		8/8		20– 640
	10^{-4}	1/3	3.9	3/3	6.7 or >	80– 640
	10^{-5}	1/9		9/9		160–1280
	10^{-6}	0/7		6/7		40– 640
Laboratory systems—					ID_{50}†	
Chorio-allantoic membrane					8.9	
Rabbit kidney cell cultures					8.5	
Monkey " " "					8.7	

* No. positive/No. tested.

† Expressed as the dilution $\log_{10}$/1.0 ml of vaccine except in scarification group where titers are uncorrected.

‡ Reciprocal of serum dilution.

tially identical, $10^{-2.6}$ and $10^{-2.4}$ respectively. Following jet inoculation, the CID_{50} was about 10-fold higher than that seen following scarification. The serologic results, however, were markedly different in that the IID_{50} of the vaccine was calculated to be $10^{-6.7}$ or greater. In fact, 6 of 7 children jet-inoculated with the highest dilution of vaccine experienced sero-conversion. Considering the laboratory assay data appearing in Table II, it is evident that the IID_{50} of smallpox vaccine in sero-negative individuals following jet inoculation approaches the titer of the vaccine as expressed in ID_{50} for cell cultures or egg membranes.

The antibody response to jet-injected vaccine was quantitatively similar to that observed after successful percutaneous vaccination. However, there was no correlation between antibody level and cutaneous reactivity. The geometric mean titer (GMT) of antibodies in the 18 persons who responded serologically to jet inoculation but who failed to develop skin lesions was 1:332 as compared to 1:151 for the 12 jet-vaccinated children who developed typical vesicles. All vaccinees experiencing sero-conversion after percutaneous inoculation exhibited primary lesions; the GMT of this group was 1:178.

Discussion. About one million West African children have been jet-inoculated with live measles vaccine and it is likely that automatic jet injectors will continue to be used extensively in Africa and other developing areas. Thus, the data presented are pertinent, indicating that the potency of live measles vaccine is unaltered by jet inoculation.

Inoculating susceptible children by conventional techniques, earlier workers reported that experimental live measles vaccines were about 10-fold more infectious for man than for HEp-2(9) and HeLa(10) cell cultures. Our findings extend this general experience but indicate that BS-C-1 cell cultures are as sensitive as the human host in detecting minimal amounts of the attenuated virus. These observations are of special interest since they demonstrate that the standard *in vitro* test system employed by the DBS for live measles vaccine assay accurately predicts the potency of the commercially licensed product in man.

Elisberg, using one of the early experimental jet injectors, observed that most susceptible children inoculated with a potent preparation of vaccinia virus developed primary skin lesions and immunologic responses similar to those seen after percutaneous vaccination(4). The current report as well as earlier studies from this laboratory(1) confirms his observations. In our experience it has been

possible to evoke the responses classically associated with primary vaccination in 253 of 254 sero-negative children jet-inoculated with 1:50 or 1:100 dilutions of vaccines of both avian and calf lymph origin. The actual inoculum in each instance contained 10^6 to $10^{6.6}$ CAM ID_{50} of vaccinia virus, which indicates that this amount of jet-injected vaccine regularly produces primary vaccinal reactions. The present study clearly indicates that lesser quantities of virus evoke skin responses with decreasing frequency but seem to be highly effective in stimulating antibody production.

Numerous reports can be found dealing with parenteral inoculation of smallpox vaccines with syringe and needle(4,11-15). However, many of these studies do not provide definitive information concerning the potency of the vaccines used. Most agree that skin reactions are rarely associated with subcutaneous inoculation and may not be entirely typical after intracutaneous injection of vaccine. Some workers feel that the development of a local vesicular lesion is a prerequisite to the acquisition of immunity(12). Indeed, primary percutaneous vaccination is almost invariably followed by the development of HAI and virus neutralizing antibodies(16). However, it is difficult to evaluate the role of circulating antibody in protection against smallpox.

Little has been written about the serologic response to parenteral smallpox vaccination, but the data available suggest that the seroconversion rates and antibody levels in those responding are poor(4). It is of interest that in the study reported here, consistently good antibody responses were obtained in the absence of cutaneous reactivity after jet inoculation of minimal quantities of virus. It should be emphasized that jet inoculation results in a cone-shaped dispersion of inoculum into intradermal and subcutaneous tissues (17), thus, is not strictly comparable to other methods of parenteral injection. The observation that the immunologic ID_{50} of jet-injected smallpox vaccine in man is at least 10,000 times greater than the corresponding percutaneous endpoint may prove to be of practical consequence. In addition, these data provide an interesting experimental approach to the study of the role of circulating antibody in immunity to smallpox.

Summary. Live measles and smallpox vaccines were titrated in susceptible children by jet inoculation and conventional vaccination techniques. Measles vaccine potency results in man were similar by both inoculation methods and comparable to results obtained in cell culture assays. Smallpox vaccine potency as measured by cutaneous reactivity was about 10-fold greater in jet-inoculated children than in those inoculated percutaneously. However, jet injection of high dilutions of vaccinia virus commonly resulted in sero-conversion in the absence of a local skin lesion.

The authors gratefully acknowledge the assistance of Dr. P. Carrié, Dr. H. Lartigue, Dr. F. Lebreton, Mrs. Ethel D. Rosenblatt and Mr. Raymond L. Vaughn in conducting this study.

1. Meyer, H. M., Jr., Hostetler, D. D., Jr., Bernheim, B. C., Rogers, N. G., Lambin, P., Chassary, A., Labusquière, R., Smadel, J. E., Bull. World Health Org., 1964, v30, 783.

2. Enders, J. F., Katz, S. L., Milovanovic, M. V., Halloway, A., New England J. Med., 1960, v263, 153.

3. Rosen, L., Virology, 1961, v13, 139.

4. Elisberg, B. L., McCown, J. M., Smadel, J. E., J. Immunol., 1956, v77, 340.

5. Meyer, H. M., Jr., Brooks, B. E., Douglas, R. D., Rogers, N. G., Amer. J. Dis. Child., 1962, v103, 457.

6. Jackson, E. B., Ley, A. C., Binn, L. N., Smadel, J. E., J. Immunol., 1956, v77, 332.

7. Hopps, H. E., Bernheim, B. C., Nisalak, A., Tjio, J. H., Smadel, J. E., ibid., 1963, v91, 416.

8. Lennette, E. H., in Diagnostic Procedures for Virus and Rickettsial Diseases, 2nd Ed., Am. Public Health Assn., New York, 1956, p46.

9. McCrumb, F. R., Jr., Kress, S., Saunders, E., Snyder, M. J., Schluederberg, A. E., Am. J. Dis. Child., 1961, v101, 689.

10. Markham, F. S., ibid., 1962, v103, 437.

11. Rivers, T. M., Ward, S. M., J. Exp. Med., 1935, v62, 549.

12. Henderson, R. G., McClean, D., J. Hyg., 1939, v39, 680.

13. Roberts, B. E., J. Prev. Med., 1932, v6, 453.

14. Yaoi, H., Jap. J. Exp. Med., 1936, v14, 311.

15. Gallardo, E., Sanz, J., Presse méd., 1937, v45, 139.

16. McCarthy, K., Downie, A. W., Bradley, W. H.,

J. Hyg. Camb., 1958, v56, 466.
17. Warren, J., Ziherl, F. A., Kish, A. W., Ziherl, L. A., J. Am. Med. Assn., 1955, v157, 633.
Received July 16, 1964. P.S.E.B.M., 1965, v118.

Sex Dependent Protein Component of Rat Liver.* (29754)

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Bond(1) has reported the presence of a sex-associated protein component in extracts from livers of male rats and absent in the extracts from livers of females. The response of this liver-protein fraction to castration and treatment of animals with estradiol or testosterone has also been described(2). Initial analytical data indicate that the sex-associated protein fraction contains a major single component and 2 minor components(2). This report confirms the initial observations of Bond with respect to the difference observed in male and female liver extracts and the influence of estradiol or testosterone treatment. The results agree well though different levels and times of hormone treatment were used. We have also studied the effect of progesterone treatment on the level of this sex-associated protein fraction in liver extracts.

Methods. Male and female Sprague-Dawley rats were received at the age of 14 days (average weight, 20 g). Immature animals used were 14 days old. Gonadectomy was done on the same day. Hormones were administered by intramuscular injection into the respective groups of animals twice a week for 13 weeks. Amount of hormone per injection was 2.5 mg testosterone propionate, 0.25 mg 17-Beta estradiol and 5 mg progesterone. All animals were sacrificed by decapitation 108 days after the initial injection. Livers were perfused with Locke's solution, homogenized, and the 105,000 $\times$ *g* supernatants prepared, according to the methods of Anderson (3). Chromatography of the supernatants was performed as described by Bond with DEAE cellulose (Carl Schleicher & Schuell Co., Type 20), equilibrated with 0.01 M Tris-chloride buffer, pH 8. Proteins were eluted with the buffer in 3 ml fractions at 1.5 ml/minute. Optical densities of the fractions were read at 280 mμ in a Beckman DU spectrophotometer.

Results. The typical chromatogram for a liver supernatant from a normal adult male is shown in Fig. 1. The sex-associated peak appears between 140-230 ml of effluent. As shown in Fig. 1, the supernatant from livers of immature males does not show this peak. The chromatograms obtained with liver supernatant from adult males 93 days after castration show a demonstrable but greatly reduced amount of protein in this area (Fig. 1). Bond has also shown that after castration the concentration of the protein was reduced to one-half of normal values(2).

Bond has shown that results from Kjeldahl nitrogen determinations compare favorably with the spectrophotometric measurements of the amount of material in the area of the sex-associated protein and that these areas can be used to assess the amount of protein present(2). Our data confirm that the concentration of the protein in the 105,000 $\times$ *g* supernatant from adult male liver is approximately 3%

Fig. 2 contrasts the chromatograms obtained from livers of normal adult males and females, and shows the absence of any protein peak in the corresponding area in the pattern obtained from livers from female rats.

When intact or castrated females are injected with testosterone propionate, the protein peak appears in the chromatograms (Fig. 3). After a single injection of 10 mg testosterone propionate, Bond reported a

* Supported by a grant made to the Training Program in the Physiology of Reproduction by the Population Council and by G. D. Searle & Co.

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