lowing vaccination with fowl pox virus on 1 April. The results of the 1955 and 1956 tests are presented in Table 1.

These data reveal that, in 1955, more than 3 times as much parthenogenesis was encountered in eggs laid after vaccination with chicken pox virus than was found in eggs laid by the same birds prior to this time ($\chi^2 = 33.0$, degrees of freedom = 1, $P < 0.001$). It is interesting to note that the milder pigeon pox virus was less effective in inducing parthenogenesis ($\chi^2 = < 1$, degrees of freedom = 1, $P < 0.35$). In the 1956 tests involving fowl pox vaccine, the difference was even greater, more than 9 times as much parthenogenesis appearing in eggs of the same birds following vaccination than was found prior to the introduction of the virus ($\chi^2 = 168$, degrees of freedom = 1, $P < 0.001$). The highest incidence of parthenogenesis was encountered in eggs laid 30 to 60 days after the birds had been vaccinated. Facilities were not available for the maintenance of nonvaccinated birds during the full term of these tests. However, data on the incidence of parthenogenesis in turkeys does not show any appreciable seasonable variation (*3*).

A further indication that fowl pox vaccine may be involved in the initiation of parthenogenesis is furnished by data obtained in 1956 with turkey eggs. In these tests a total of 3110 eggs laid by two groups of turkeys were examined for parthenogenesis over a period of 3 months.

One group was composed of 16 nonvaccinated birds, the other of 49 turkeys that had been vaccinated for fowl pox at 7 weeks and again at 30 weeks of age. Since the ancestry of each bird was known, it was possible to select birds for these tests so that in each group full sisters would be represented. The 16 nonvaccinated turkeys, representing 12 families, were housed in wire cages within a screened building where they could be kept isolated from other birds. The 49 vaccinated birds, representing the same 12 families, were kept in turkey houses where they were in direct contact with other vaccinated birds. All birds involved in these tests were virgins, having been segregated from males at 4 weeks of age, and all received the same type of feed.

A total of 738 eggs was produced by the 16 nonvaccinated turkeys during the 3-month test period. Of these, 180, or 24.4 percent, showed parthenogenetic development when they were examined following a 9- to 10-day period of incubation. Membranes only were found in 144; 47 showed blood formation in addition to membranes; and 19 eggs contained well-formed embryos.

The 49 vaccinated females produced a total of 2362 eggs during the same period. Parthenogenetic development occurred in 750, or 31.8 percent, of these eggs.

Table 1. Incidence of parthenogenetic development found in eggs of Dark Cornish chickens before vaccination and following vaccination with pigeon pox and fowl pox vaccine.

Item	1955		1956
Before vaccination			
Number of birds on test	13	29	35
Number of eggs tested	497	1190	1294
Number of eggs showing parthenogenetic development	9	12	21
Percentage of eggs showing parthenogenetic development	1.8	1.0	1.62
After vaccination with	*Pigeon pox*	*Fowl pox*	*Fowl pox*
Number of birds on test	13	29	35
Number of eggs tested	808	1653	1675
Number of eggs showing parthenogenetic development	23	53	266
Percentage of eggs showing parthenogenetic development	2.9	3.2	15.9

The parthenogenetic development was classified as follows: 548 eggs contained membranes only; 100 contained blood in addition to membranes; and 102 contained well-formed embryos.

The records of these two groups of full sisters reveal that a significantly higher percentage of parthenogenesis occurred in the eggs laid by the vaccinated group of turkeys, 31.8 percent as compared with 24.4 percent of total eggs tested ($\chi^2 = 12.3$, degrees of freedom = 1, $P < 0.001$) (*4*). This increased incidence of parthenogenesis in the eggs of the vaccinated group was evident in each of the three categories listed—that is, membranes, blood formation, and embryos.

The results secured in the foregoing tests indicate that some agent, possibly of a physiological nature, had some part in initiating parthenogenetic development. When this agent was present at, or near, the optimum level in the blood stream of genetically susceptible hens, the development initiated tended to proceed further and show a higher degree of organization. The agent, whatever its nature, possibly may be transmitted from parent to offspring through the egg, since, in eggs of both nonvaccinated groups of chickens and turkeys, a considerable percentage of eggs was encountered showing parthenogenetic development. This was true, even though these nonvaccinated females were isolated from other birds, and every attempt had been made from time of hatching to keep them disease-free.

It would appear, therefore, that at least two conditions are necessary before an advanced type of parthenogenesis occurs. First, a susceptible strain of birds is necessary. This implies not only that birds produce readily activated eggs but also that the parthenogenetic cells possess a sufficiently high inherent viability to survive until such time as the eggs are placed in an incubator. In this sense the eggs produced by most of our domestic breeds of chickens cannot be considered as susceptible, since in nearly every instance the parthenogenetic cells have died by the time the eggs are laid. The second condition is that an activating agent must be present in the blood stream. When this agent is present at, or near, an optimum level, it has the effect of inducing parthenogenetic development.

It is not known, at present, whether the fowl pox virus as such is the sole agent initiating parthenogenesis or whether some contaminant which may be present in the vaccine is also involved. Neither is it known just how this virus may exert its effect, whether it is a direct or an indirect one.

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Spontaneous Activity in Denervated Insect Muscle

Following section and peripheral degeneration of the motor nerve supply, insect muscle generally has been thought to become inactive, even in response to direct electric stimulation (*1*). Recent physiological investigations of the thoracic spiracle muscles of cockroaches, *Periplaneta americana* (L.), have demonstrated a strikingly different mode of behavior, which is deemed of sufficient interest to the general question of the irritability of insect muscle to warrant preliminary description.

For each thoracic spiracle of the American roach there is a single occlusor

muscle which acts against the elasticity of the spiracle lips; hence, it remains continuously under tension. An experimental analysis of the innervation of these spiracles has demonstrated that they receive motor innervation from the median nerves (*2*). In more than 200 instances, median nerve section has prevented the spiracle from cooperating in the ventilative rhythm. Nevertheless, the spiracle muscle can remain contracted after complete denervation and will open in response to relatively high concentrations of CO_2. Spiracle movements for the first 3 to 5 days following denervation, although no longer coordinated with the ventilative pattern, appear otherwise normal; muscle twitches are rapid and involve the whole muscle. During subsequent days these contractions become slower and are produced by asynchronous activity of parts of the muscle. This behavior, which perhaps should be called fasciculation, continues unabated for at least 90 days or until the muscle is reinnervated by the regenerating median nerve.

At any time during this period the muscle may be removed to a dish of saline where it will continue to fasciculate. Surprisingly, during this entire period the muscle remains responsive to electric stimulation. Although the rheobase varies from 1.8 to 4.0 volts there appears to be no consistent variation with time since denervation. Electric stimuli do not interfere with the spontaneous fasciculative rhythm at low stimulus frequencies, but tetanic contractions are induced by liminal stimuli at about 20 per second.

A study of methylene blue preparations indicates that the onset of fasciculation occurs at about the same time that the peripheral branches of the sectioned median nerve disappear.

The differences between the behavior of denervated thoracic spiracle muscles and that described for other insect muscles are not yet understood and currently are under investigation. One possible source of the difference is that the spiracle muscle is continuously under tension. Consequently its mechanical arrangements are rather similar to those of some insect flight muscles, in which one set of muscles contracts against the tension of another, or to the dipteran haltere muscle, which contracts in opposition to the elasticity of the haltere articulation. In both the dipteran flight and the dipteran haltere muscles contraction is not necessarily the direct consequence of the motor impulse (*3*). Rather, the motor impulse serves to increase sensitivity of the muscle to stretch to such a degree that a twitch occurs. By analogy, it seems possible that fasciculation of the denervated spiracle muscle is due to increased sensitivity to stretch. This is supported by the observation that a denervated muscle which has become momentarily quiescent may be readily started fasciculating again by mechanically opening the spiracle. A corollary hypothesis that the presumed stretch sensitivity of denervation involves increased muscle sensitivity to the neuromuscular mediator substance is currently under investigation.

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Nicotinic and Glutamic Acids, Nicotinamide, and Glutamine in Cigarette Tobacco Smoke

During the course of studies of the constituents in tobacco smoke (*1*) it became of interest to investigate the presence of some naturally occurring, biologically active nitrogen compounds that had not previously been reported in the literature. The presence of the amino acids, glutamine and glutamic acid, and of nicotinic acid and nicotinamide are reported in this paper.

Cigarettes, 70 mm in length, were smoked according to a standard procedure reported elsewhere (*2*). An amphoteric fraction of the collected smoke containing nitrogen and giving a positive reaction to ninhydrin was chromatographed on Dowex-50 (8 percent cross-linked, 200 to 400 mesh, H^+ form), and six separate ninhydrin-positive fractions were eluted with 2*N* HCl. The fractions were compared directly with authentic samples of amino acids in different paper chromatographic solvent systems. Table 1 shows the close agreement of fracions 1 and 2 with glutamic acid and glutamine, respectively. After heating fraction 2 in 1*N* HCl for 1 hour at 100°C and rechromatographing, it was found that the spot which initially migrated exactly like authentic glutamine no longer existed. Instead, a new spot appeared which migrated parallel with known glutamic acid. The conversion after acid hydrolysis of fraction 1 into glutamic acid is additional evidence that fraction 1 was originally glutamine.

A quantitative determination that utilized the color developed by the reaction of the amino acids with ninhydrin showed that the smoke from a 50/50 blend of bright and burley tobaccos contained, respectively, 10 and 7 μg of glutamic acid and of glutamine per cigarette. The four remaining ninhydrin-positive amphoteric substances have not been identified.

The migration of the pyridine alkaloids and of nicotinic acid and nicotinamide in several solvents on buffered paper has been shown to be a function of the *p*H of the buffer (*3–5*). The pattern of movement of a given pyridine-containing compound in the system has been useful in its characterization (*4*). Nicotinic acid has been tentatively identified in tobacco smoke by use of such a procedure (*5*).

For a verification of the presence of nicotinic acid and a quantitative determination of the acid and of nicotinamide, the following procedure was followed. Smoke collected in Dry Ice-acetone traps was dissolved in ether and extracted three times with equal volumes of 2-percent HCl solution. The acid layer was adjusted to *p*H 10.0 with sodium hyroxide and extracted twice with an equal volume of ether to remove most of the nicotine-type alkaloids present. A check run with knowns showed that, after this treatment, all the nicotinic acid and 90 percent of the nicotinamide remained in the basic aqueous layer. This solution was then neutralized and concentrated to a small volume. An amount of solution corresponding to one cigarette was then applied in a 0.5-cm wide streak across the entire width of a dry, 20- by 40-cm Whatman No. 1 paper that had previously been soaked in *p*H 6.3 sodium citrate buffer.

On a separate 5- by 40-cm paper also buffered at *p*H 6.3, 20 μg of a known mixture of equal parts of acid and amide were applied and run simultaneously with the paper chromatogram containing the cigarette smoke. The R_f values of the acid and of the amide on the pilot paper were 0.30 and 0.80, respectively. The values were determined by developing a color reaction with *p*-aminobenzoic acid in an atmosphere of cyanogen bromide.

Two pencil lines that corresponded to the R_f of the acid and of the amide as

Table 1. Comparison of R_f values of glutamic acid and glutamine with elution fractions from Dowex-50 (H^+) ion-exchange resin.

Solvent system	Glutamic acid	Fraction 1	Glutamine	Fraction 2
Phenol/water (7/3)	0.27	0.27	0.56	0.56
n-Butanol/acetic acid/water (40/10/50)	0.22	0.22	0.12	0.13
Methyl ethyl ketone/propionic acid/water (75/25/30)	0.30	0.30	0.23	0.23