the least of which is their contribution to animal production, some of which was discussed above.

Literature Cited

(1) Anderson, R. M., Hanson, L. E., Ferrin, E. F., Univ. of Minn., Dept. An. Husb. Mimeo **H-105** (Sept. 1951).
(2) Becker, D. E., Terrill, S. W., Nesheim, R. O., Meade, R. J., Univ. of Ill., Dept. An. Sci. Mimeo **AS 215** (1951).
(3) Bird, H. R., Groschke, A. C., Rubin, M., *J. Nutr.* **37,** 215 (1949).
(4) Briggs, G. M., Jr., Luckey, T. D., Elvehjem, C. A., Hart, E. B., *Proc. Soc. Exptl. Biol. Med.* **55,** 130 (1944).
(5) Carpenter, L. E., *Arch. Biochem.* **27,** 469 (1950).
(6) *Ibid.*, **32,** 181 (1951).
(7) Carpenter, L. E., Larson, Nora L., *J. Animal Sci.* **11,** 282 (1952).
(8) Conrad, J. H., Beeson, W. M., Purdue Univ., Dept. An. Husb. Mimeo **A.H. 214** (Aug. 23, 1957).
(9) Conrad, J. H., Jordan, C. E., Beeson, W. M., *J. Animal Sci.* (Abstr.), **18,** 1514 (1959).
(10) Conrad, J. H., Nelson, D. M., Beeson, W. M., Purdue Univ., Dept. An. Husb. Mimeo **AS 263** (Aug. 1959).
(11) Hanson, L. E., Carpenter, L. E., Aunan, W. J., Ferrin, E. F., *J. Animal Sci.* **14,** 513 (1955).
(12) Hanson, L. E., Ferrin, E. F., *Ibid.*, **15,** 376 (1956).
(13) Hanson, L. E., Ferrin, E. F., Anderson, P. A., Aunan, W. J., *Ibid.*, **14,** 30 (1955).
(14) Hanson, L. E., Hill, E. G., Ferrin, E. F., *Ibid.*, **15,** 280 (1956).
(15) Hanson, L. E., Hill, E. G., Meade, R. J., Univ. of Minn., Dept. An. Husb. Mimeo **H-147** (Sept. 1957).
(16) Jordan, C. E., Conrad, J. H., Beeson, W. M., Purdue Univ., Dept. An. Sci. Mimeo **AS 238** (Nov. 1958).
(17) Jukes, T. H., Stokstad, E. L. R., Taylor, R. R., Cunha, T. J., Edwards, H. M., Meadows, G. B., *Arch. Biochem.* **26,** 324 (1950).
(18) Lamana, C., *Science* **95,** 304 (1942).
(19) Luecke, R. W., McMillan, W. N., Thorp, F., Jr., *Arch. Biochem.* **26,** 326 (1950).
(20) Merck and Co., "Coccidiosis and Poultry Management," 1958.
(21) Moore, P. R., Evenson, A., Luckey, T. D., McCoy, E., Elvehjem, C. A., Hart, E. B., *J. Biol. Chem.* **165,** 437 (1946).
(22) Morehouse, N. F., *Poultry Sci.* **28,** 375 (1949).
(23) Morehouse, N. F., Mayfield, O. J., *J. Parasitol.* **32,** 20 (1946).
(24) Notzold, R. A., Becker, D. E., Adamstone, F. D., Terrill, S. W., Jensen, A. H., *J. Animal Sci.* **15,** 1234 (1956).
(25) Overby, L. R., Frost, D. V., *Toxicol. Appl. Pharmacol.* **4,** 38 (1962).
(26) Proc. "2nd National Symposium on Nitrofurans in Agriculture," Univ. of Ga., pp. 39–61 (March 27–28, 1958).
(27) Proc. "3rd National Symposium on Nitrofurans in Agriculture," Univ. of Ky., pp. 52–76 (Sept. 8–9, 1960).
(28) Stokstad, E. L. R., *Physiol. Revs.* **34,** 25 (1954).
(29) Teague, H. S., *Proc. Maryland Nutr. Conf. Feed Mfrs.* p. 17 (March 1960).
(30) Warner, D. R., Hanson, L. E., *Nebr. Agr. Expt. Sta. 64th Ann. Rpt.*, p. 105 (1951).

Received for review October 11, 1962. Accepted April 4, 1963. Division of Agricultural and Food Chemistry, 142nd Meeting, ACS, Atlantic City, N. J., September 1962. Paper No. 1130, Miscellaneous Journal Series, Minnesota Agricultural Experiment Station, St. Paul, Minn.

FEED ADDITIVES

Are Animal Feed Additives Hazardous to Human Health?

JOHANNES BRÜGGEMANN, JÜRGEN SCHOLE, and JÜRGEN TIEWS

Institut für Physiologie und Ernaehrung der Tiere, University of Munich, Munich, Germany

Recommended levels of Amprolium, zoalene, Arzene, and arsanilic acid were fed to broiler chickens for several weeks. Liver, kidney, fat, and muscle tissue of individual birds were analyzed for specific residues at regular time intervals. Contrary to the steadily increasing drug intake during the experiments, residues in the tissue remained on a constant low level—a safe indication that the drugs did not accumulate. There was no linear increase but only a slight response in the residue levels when multiple (1 to 10) drug levels were used in the feed.

Much of the confusion and uneasiness about the need for and the value of feed additives in animal nutrition today is caused by lack of understanding by the layman. Moreover, some of the information has been distorted. Efficiency in animal production has been raised to an almost unbelievable level as compared with that of 20 or 30 years ago. This leaves the layman suspicious, at least in Europe where people have not experienced as many other technical advances as have people in the United States. The layman believes that food produced with less than half the amount of feed that was needed 20 years ago will probably have only half of the nutrient value. He believes that the increasing profit in animal production is brought about by obscure and even dangerous feed additives and that the consumer pays for this by jeopardizing his health. The first approach to the supposed hazard to man of feed additives must therefore be a consideration of what actually happens in animal production today.

Economizing on Maintenance Feed

The steadily increasing feed efficiency in animal production has little to do with the use of feed additives. Most of it can be explained by our changing attitude toward animal production. Twenty years ago we were content with hens that produced an egg only every third day or 120 eggs per year. Because of better breeding and, to a certain extent, better feeding and management techniques, most hens now lay an egg every 36 hours or 250 eggs per year. This better, or much faster, performance influences feed conversion since energy cannot be lost. Just as heat energy can be transformed into mechanical energy or into electrical power, feed energy must show up in one form or another unless it is wasted in feces or urine. On the average, only 30 to 40% of the feed's net energy will show up in eggs, milk, meat, butter, or body

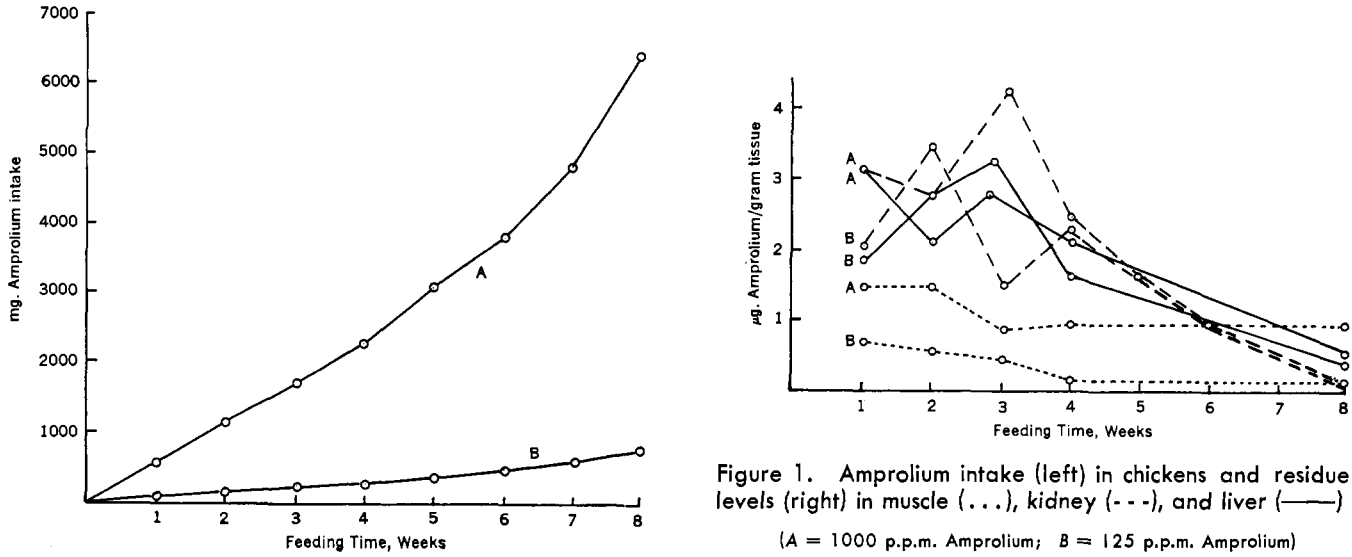

Figure 1. Amprolium intake (left) in chickens and residue levels (right) in muscle (. . .), kidney (- - -), and liver (——)

(A = 1000 p.p.m. Amprolium; B = 125 p.p.m. Amprolium)

fat. The remaining 60 to 70% is expended for the animal's maintenance or routine living requirements.

One can calculate the 24-hour maintenance requirement for energy in calories from Kleiber's formula for metabolic size (7). The smaller the animal, the higher its maintenance requirement on a liveweight base. For example, a 2-kg. laying hen will need approximately 80 grams of feed daily to sustain life. This figure depends somewhat on the composition and therefore the energy content of the ration being fed. The hen's total feed capacity, however, is limited to no more than 120 to 130 grams daily during high production periods. Therefore, only a minor fraction, roughly one third, of the feed intake is available for egg production.

One cannot arbitrarily eradicate the maintenance requirement or even decrease it. Therefore, the only way to change this unfavorable maintenance-to-production feed ratio is to keep only those animals that voluntarily work twice as hard as those of 20 years ago. This, however, is more a genetic than a feeding problem. One can see that there is a very simple explanation available for the gap in feed intake per egg, which, in 1962, was approximately 175 grams as compared with 300 grams in 1945.

Demand for Nutrients Rather Than for Traditional Feeds

In animal nutrition there is seldom a demand for specific feedstuffs. The nutritionist has learned that feed demands must be converted into nutrient demands. All animals have a nutritional need for proteins, energy sources (mainly derived from carbohydrates and fats), mineral substances, and vitamins which are present in varying quantities as "building units" in all feeds. Since there is a demand for building units rather than for individual feeds, efforts have been directed to combining nutrients in a feed ration in the most profitable way, and adapting them to the animal's nutritional requirements. As a result, feed rations today are more concentrated in nutrients than those of 15 years ago, which accounts partly for the better feed conversion. Part of the vitamins, trace elements, amino acids, or xanthophylls for egg yolk pigmentation missing in common economical feedstuffs are added to the ration to make it more effective. Feed additives of this type, added in proper amounts, cannot be hazardous to human health since they have been natural ingredients of plants and other feed sources for thousands of years.

Growth-Promoting and Disease-Preventing Agents

These agents may well be compared in significance with the discovery of vitamins in the early part of this century. Only healthy livestock will show the best performance and highest efficiency in producing meat, milk, and eggs. However, there may exist livestock "healthier than healthy," depending only on how "good health" is defined. Adding small amounts of antibiotics to the feed—begun in 1948—proved to be of apparent nutritive value. Animal performance improved the same as it would have with an additional supply of nutrients. Antibiotics, however, cannot be called nutrients per se. They put healthy-appearing livestock into an even more healthy state by cutting down detrimental effects originating from the animal's environment—mainly from bacteria most commonly spread in the intestine—thus saving energy for meat production that otherwise would be used in the animal's "defense budget." The nutritive effect of antibiotics, therefore, may be defined as the better performance and better feed conversion achieved by making an animal "healthier than healthy."

Other pharmaceuticals in feeds such as coccidiostats, anthelmintics, and also some arsenicals act principally the same way. The "growth-promoting" or, even more pertinent, "growth-permitting" properties of antibiotics and arsenicals are actually the consequence of their disease-preventing qualities, although their mode of action is still under discussion. Other feed additives—tranquilizers and estrogens—cut down the high metabolic rate and so shift some of the energy needed for body maintenance to the production of meat and eggs. They, too, cannot be considered hazardous for human health, provided excretion or metabolic destruction of these compounds by the animal is sufficiently complete.

Possible Side Effects of Feed Additives

There are several possibilities that have to be considered. Allergic reactions from handling may be one. In Germany, for instance, penicillin has been charged with this side effect, but the only allergies so far have resulted from the use of this antibiotic in human or animal therapy.

Development of bacterial resistance to a feed additive may be another side effect. This may diminish the therapeutic effectiveness of the compound if it is used simultaneously in the medical field. Bacterial resistance, however, has been observed only if antibiotics exceed the "nutritive" level in the feed. Even then resistance returns to normal when the compound is removed from the ration.

A feed additive may have a detrimental effect on the quality of meat, eggs, and butter by changing the normal composition. Thyrostatics are said to cause a high moisture content in meat, but this has not been conclusively confirmed. There are numerous questions that re-

quire careful examination before a new feed additive may be used safely. In this paper, only possible carry-over effects —the residue problem in human food— are considered.

Do Feed Additives Accumulate in Animal Tissue?

This possibility will have to be studied carefully for every new compound before its release, since reliable predictions cannot be made without experimental data. So far, however, the only feed additives known to accumulate are the common nutrients such as vitamins and minerals. Vitamins A and D are stored in the animal's liver or kidney. Vitamin E is stored in the fat tissue rather ubiquitously. Carotenoids like lutein and other related compounds as well as various vitamins will be deposited in egg yolk. Trace elements such as copper and iron may be stored in the liver. A systemic or selective storage could not be established, however, for other currently used feed additives, mostly pharmaceutical agents or hormonelike compounds.

It is interesting therefore to speculate as to why feed additives other than naturally occurring feed ingredients show little tendency to be stored in animal tissue. One is inclined to say that animals are designed for the utilization of nutrients. Vitamins and minerals may be saved and stored for the animal's own welfare. But many other chemical compounds are either useless, or even poisonous. There would be no reason to store such compounds. It therefore seems logical to assume that the animal in protecting itself will try to excrete or metabolize such material as quickly as possible. Otherwise, it would suffer on feed additives in a comparatively short time. Very often a feed additive—for example, zinc bacitracin—is not even absorbed to a great extent. It acts in the intestine only, and the main portion of the additive leaves the body without having entered the blood stream.

Table I. In Vitro Destruction of Amprolium in Chick Tissue (Broiler, 2 Kg.)

(20 μg. of Amprolium added per gram of tissue)

% Amprolium Regained[a] after Incubation (37° C.)

		Pretreatment: 1250 P.P.M. Amprolium in Feed					
		0 Weeks		2 Weeks		3 Weeks	
Hours	Blank	Muscle	Liver	Muscle	Liver	Muscle	Liver
0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
2	99.5	98.5	94.5	100.0	80.0	88.0	94.3
4	97.7	98.5	88.8	98.3	73.2	89.8	82.7
6	95.6	98.5	91.5	97.5	71.0	88.2	55.7

[a] Fluorometric determination.

Although accumulation has not been observed with feed additives commonly used today, exceptions may exist. Fluorine and lead accumulate in bone tissue. They are sparingly absorbed, but may be even more slowly excreted. Lead mobilization under conditions of stress may follow years of such storage, thus leading to lead poisoning (*11*). Another exception became known with the use of chlorinated hydrocarbons as insecticides such as dichloro-diphenyl-trichloroethane (DDT), which has now been replaced by a safer methoxychlor compound. However, these insecticides have never been voluntarily added to feed rations. They have not been presented for approval by health authorities nor are they part of the animal nutrition program. They are considered undesirable feed contaminants; nevertheless, they formed the psychological background for public uneasiness and fear about feed additives.

Whether or not one considers storage of a feed additive as an exception, the possible accumulation of feed additives in different tissues, their absorption and excretion rates, and, if possible, their metabolic pathways must be investigated carefully. Normally, excreta will carry most of a feed additive or its metabolites out of the body; but eggs and milk may also carry traces of feed additives. Both must receive special attention.

Residue Levels in Tissue

Many experiments on residue levels in tissue have been run in different countries in recent years (*1, 4, 5, 8-10*). Sometimes it is difficult to explain why residues are found in the tissue during the time a compound is fed and absorbed. This is

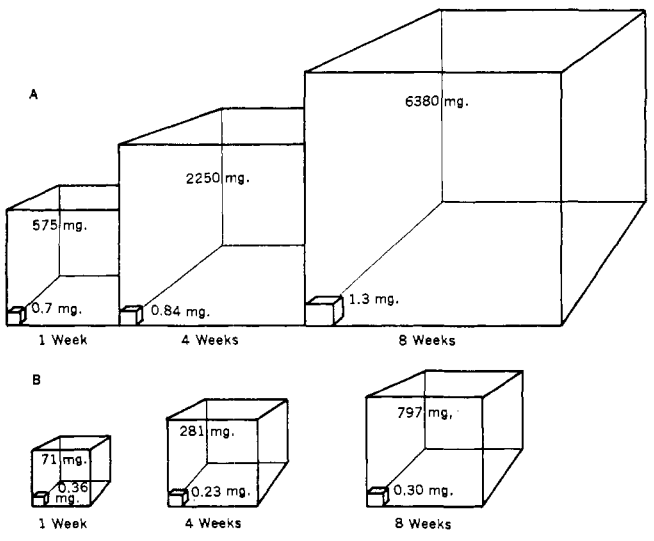

Figure 2. Relationship of total intake (large cubes) to total residue (small cubes) of Amprolium in chickens

(*A* = 1000 p.p.m. in feed; *B* = 125 p.p.m. in feed)

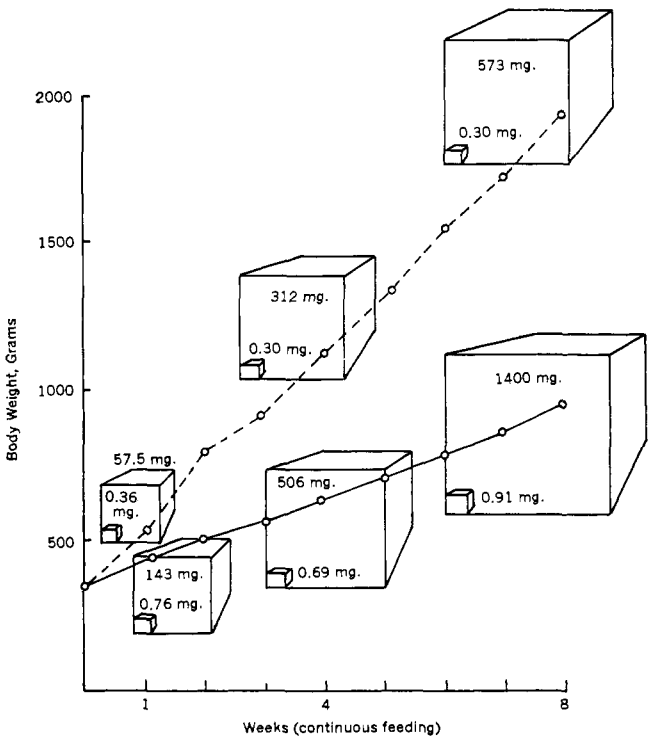

Figure 3. Relationship of total zoalene intake (large cubes) to total zoalene + ANOT residues (small cubes) in chickens

(– – – –, 125 p.p.m. zoalene in feed; ——, 625 p.p.m. zoalene in feed)

Table II. In Vitro Destruction of Zoalene in Chick Tissue (Broiler, 1 Kg.)

(400 μg. of zoalene added per 20 grams of tissue)

		μg. Zoalene and ANOT per 20 Grams Tissue Regained after Incubation (37° C.)											
		Pretreatment: 312 P.P.M. Zoalene in Feed											
		0 Weeks				1 Week				8 Weeks			
		Muscle		Liver		Muscle		Liver		Muscle		Liver	
Hours	Zoal. Blank	Zoal.	ANOT	Zoal.	ANOT	Zoal.	ANOT	Zoal.	ANOT	Zoal.	ANOT	Zoal.	ANOT
0	360	414	16	410	46	483	11	320	50	378	22	388	44
2	...	...	...	19	276	460	61	284	115	...	...	...	...
4	...	...	...	...	...	...	...	...	...	137	123	108	154
5	...	307	136	<0.1	284	293	87	284	114	...	...	...	...
8	380	35	294	<0.1	287	17	218	18	312	37	310	18	314

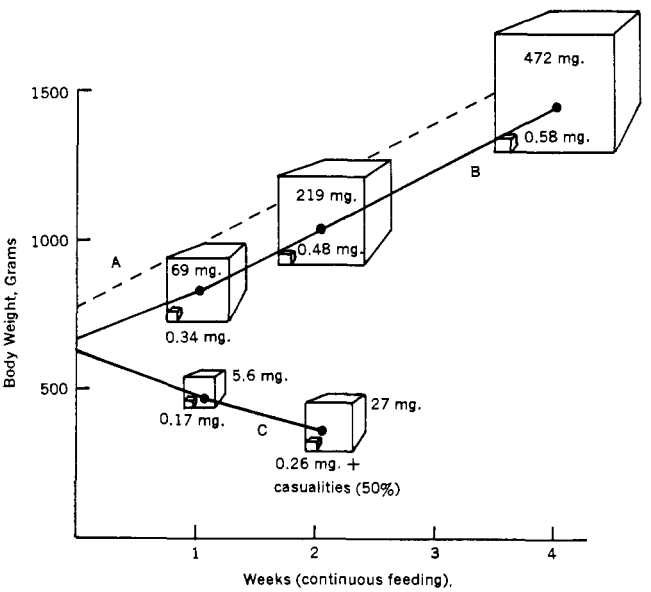

Figure 4. Relationship of total intake (large cubes) to total residue (small cubes) of Progen and Arzene in chickens

(A = control; B = 500 p.p.m.; C = 200 p.p.m.)

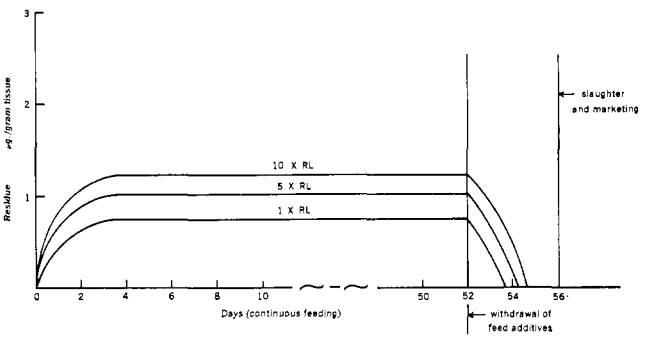

Figure 5. Typical residue curve of feed additives in soft tissue of broiler chickens

(RL = recommended level of additive in feed, p.p.m.)

normal, however, and the tissue will "dry off" these residues when the feed additive is removed from the ration (Table III).

Another point of controversy is the fact that the residue level in the tissue may increase somewhat if the level of feed additive is raised. Sometimes even this is considered a form of residue accumulation since a certain confusion exists about the maximum residue level that can be tolerated safely. Some authorities advocate the zero-level even though a zero-value will depend entirely on the sensitivity of the analytical method used.

Experimental Data

The following figures deal with the residue problem after certain coccidiostats were fed to chickens. They may be considered as typical for any feed additive used today.

Figure 1 shows Amprolium [1-(4-amino - 2 - *n* - propyl - 5 - pyrimidinylmethyl) - 2 - picoliniumchloride hydrochloride] residues found in different tissues of birds continuously fed one (125 p.p.m.) and 8 (1000 p.p.m.) times the recommended level. A certain accumulation of Amprolium in the microgram range could be observed during the first 3 weeks of feeding, but then the residue level decreased. This may be explained by more intensified degradation or excretion of the Amprolium molecule due to better adaptation, mainly of the liver, during a long feeding period (Table I).

Figure 2 shows that there is no accumulation of residues in the tissue after Amprolium feeding at even eight times the recommended dose. The experimental data are the same as in Figure 1, but this type of plotting illustrates better the capability of an animal to eliminate Amprolium from its tissues. No growth depression or other abnormalities could be observed in these experiments.

Figure 3 represents similar data for zoalene, another effective and modern coccidiostat used in the poultry industry. Five times the recommended level of zoalene in the feed depressed the growth of the birds significantly, indicating that this compound is somewhat less tolerated by the animal. Birds receiving 2.5 times (312 p.p.m.) the usual level of zoalene in the feed grew as rapidly as the control, however. But again, in none of the groups could an accumulation of zoalene in the tissue be observed. The tiny amounts of zoalene and Amprolium in these figures represent an equilibrium between drug intake and drug excretion. Within the limits used here, the animal is able to adjust itself to higher levels of drug intake without storing any of the surplus. There was no linear increase of residues in the tissue, but only a slight response when multiple levels of the drug were fed. The data seem to indicate that this response follows a logarithmic function (*10*). The slightly increased drug content of the tissue with higher level of drug intake, therefore, should not be interpreted as storage of the drug in the tissue. It indicates only the somewhat higher amount of drug "passing" the tissue at the time of slaughter because of the higher level of drug in the feed.

Zoalene (3,5-dinitro-*o*-toluamide) can easily be transformed into ANOT (3-amino-5-nitro-*o*-toluamide). Again, the tissue seems to become accustomed to the degradation process during a longer feeding period (Table II). However, these data are not so striking as those for

Table III. Residues (μg. per Gram) of Zoalene and ANOT after Withdrawal

Days after Withdrawal	Muscle		Liver	
	Zoal.	ANOT	Zoal.	ANOT
	PRETREATMENT: ZOALENE, 125 P.P.M. IN FEED FOR 8 WEEKS			
0	1.6	0.9	0.3	2.5
1	<0.1	0.2	<0.1	1.0
2	<0.1	<0.1	<0.1	<0.1
	PRETREATMENT: ZOALENE, 312 P.P.M. IN FEED FOR 8 WEEKS			
0	1.3	1.1	0.3	3.1
1	<0.1	0.2	<0.1	<0.1
2	<0.1	<0.1	<0.1	<0.1

Amprolium in liver tissue. How quickly the last trace of residue will disappear from the tissue after the removal of zoalene from the feed can be seen in Table III. Similar data are at hand for Amprolium.

Organic arsenicals showed identical behavior (Figure 4). The more toxic Arzene (arsenosobenzene) which contains trivalent As in an organic molecule killed 50% of the animals at 10 times the recommended level in 2 weeks. All growth was inhibited, but there was no marked accumulation of As in the tissue. The less toxic arsanilic acid (Progen) which contains pentavalent As was tolerated well even at the high level used in the feed. Although the total drug intake increased daily, the residue level in the tissue remained low. Once the compound was removed from the feed the tissue "dried off" quickly.

Figure 5 represents typical residue curves of feed additives in animal tissues. There is not a single feed additive known to the authors and used today in animal nutrition that would not follow this pattern. Experimental studies are at hand in the authors' laboratories for nitrofurazone, nitrophenid, and nicarbazin, as well as other coccidiostats. This is true also for antibiotics in feeds, as checked by the authors for aureomycin, terramycin, penicillin, and bacitracin, so long as the recommended and maximum level of 100 p.p.m. in the animal's tota ration is not exceeded. In higher concentrations, tetracyclines show a tendency for deposition in bone material but not in the soft tissue of the animal's carcass (*2, 3, 6*).

Figure 5 also demonstrates that tiny amounts of residues are unavoidable at the time of feeding as long as the feed additive will be absorbed from the intestine. The residue level will increase only slightly when the drug level in the feed is raised within certain limits. Contrary, however, to the steadily increasing total drug intake during longer feeding periods, residues in the tissues remain on a constant low level. Sometimes they even decrease, as in the case of Amprolium. Metabolites as degradation products of the drug should always be considered during analysis. Best information will often be obtained with labeled compounds.

Since livestock withstand several thousand times the drug level ever expected in human food derived from those animals, there is little or no chance for residue traces to jeopardize human health. There is no valid reason to assume that those traces will not be excreted by man as they are by millions of farm animals. This practical experiment, therefore, is of much greater significance in studying the safety of a feed additive than toxicity studies on rats, mice, cats, and dogs, performed only once or twice in a laboratory, could ever be.

Literature Cited

(1) Bevirt, J. L., Thiegs, B. J., *Poultry Sci.* **40,** 1335 (1961).
(2) Buyske, D. A., Eisner, H. J., Kelly, R. G., *J. Pharmacol. Exptl. Therap.* **130,** 150 (1960).
(3) Dessau, F. J., Sullivan, W. J., *Toxicol. Appl. Pharmacol.* **3,** 654 (1961).
(4) Frost, D. V., *Nutrition Revs.* **18,** No. 5, (1960).
(5) Frost, D. V., Overby, L. R., Spruth, H. C., J. AGR. FOOD CHEM. **3,** 235 (1955).
(6) Kelly, R. C., Buyske, D. A., *J. Pharmacol. Exptl. Therap.* **130,** 144 (1960).
(7) Kleiber, M., "The Fire of Life," Wiley, New York, 1961.
(8) Ott, W. H., Dickinson, A. M., Iderstine, A., *Poultry Sci.* **39,** 1280 (1960).
(9) Overby, L. R., Frost, D. V., *Toxicol. Appl. Pharmacol.* **4,** 38 (1962).
(10) Polin, D., Porter, C. C., Wynosky, E. R., Cobb, W. R., *Poultry Sci.* **41,** 372 (1962).
(11) Stewart, C. P., Stolman, A., "Toxicology," Academic Press, New York, 1960.

Received for review October 11, 1962. Accepted April 1, 1963. Division of Agricultural and Food Chemistry, 142nd Meeting, ACS, Atlantic City, N. J., September 1962.

FEED ADDITIVES

The Additives Amendment in Practice

R. W. ENGEL

Department of Biochemistry and Nutrition, Virginia Polytechnic Institute, Blacksburg, Va.

THE FAMOUS "poison squad" experiments of the Wiley era in which young men served as test subjects created the misconception that all chemicals are harmful and the related idea that any amount of a chemical is poisonous (*6*). Thus, a struggle has been going on for over half a century between research and reason on one side and, on the other side, the emotional concept that all additives are harmful. The proponents of research and reason have included the land grant institutions, the U. S. Department of Agriculture, and the reputable food industry. It is comforting to realize that these proponents have generally prevailed over their adversaries who reiterated the misconceptions of the Wiley era. As a result, we are today enjoying a safe, wholesome food supply.

The 1938 revision of the original Pure Food Law probably constituted one of the finest pieces of legislation governing foods ever enacted into law anywhere in the world. The 1938 law gave broad authority to regulate the labeling of foods claiming special nutritive benefits.

In practice, the 1938 law had one flaw which Congress felt needed to be remedied—namely, it prohibited the addition in any quantity of any poisonous or deleterious substance to foods except where it was required or could not be avoided in food production. This particular flaw, it was widely felt, could not afford complete protection to the consumer, because prior establishment of the safety of an additive was not required. Furthermore, the flaw in the 1938 law also tended to retard technological advances in the food-processing field, since, any reputable manufacturer of chemicals, or any producer or processor of foods could not consider the use of a substance at any level if it was demonstrated to be poisonous at high levels, even though at low levels it might be safe and serve a useful purpose (*2*).

Prior to the enactment of the 1958 amendment to the Food, Drug, and